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Provenancing Humans: The Real Uncertainty of Stable Isotope Data

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Abstract

Stable isotope profiling is a relatively new technique within the field of human identification. It allows forensic investigators to deduce information pertaining to an individual's geographical provenance, recent movements, and even dietary intake. Human identification requires the development of new methods able to overcome the issues associated with traditional techniques such as the degradation of DNA samples in aqueous environments, and the extreme fragmentation of skeletal material. Stable isotope profiling is a rapid, cost effective, and accurate technique capable of assisting forensic investigations by focussing resources and providing additional information to the biological profile provided by the anthropologist.

Variation in the isotopic composition of local tap water arises as a result of mass-dependent fractionation processes occurring within the hydrologic cycle. These processes occur constantly as water is transported around the globe, and produces distinct isotopic signatures for tap water depending upon geographical location. The isotopic content of tap water is then incorporated into human body tissue via dietary intake, with further fractionation occurring as a result of metabolic processes. Variation in both metabolic rates within the tissues of the same individual, and of tissues belonging to different individuals, will result in differences in the isotopic composition of human material. However, there are very few data available demonstrating intra- and inter- individual variability. This information is of particular use in forensic investigations, as judges will often evaluate the errors associated with a technique before declaring evidence admissible. Barristers also use these data to support or interrogate the statements provided by individuals involved in a case.

The primary aim of this research is to quantify the inter- and intra-individual variation associated with human tissue, in particular femoral material. This was achieved by collecting femoral sections from cadavers, and analysing the ^{13}C and ^{18}O content of the carbonate portion. The data collected from this research suggested there is significant variation in the isotopic variability of $\delta^{18}O$ both within, and between individuals. It also

indicated that there was no significant difference between the isotope values obtained from the left and right femora of the same individual, however there was significant variation between a number of samples originating from the same piece of femur. It was possible to link the $\delta^{18}O$ values obtained from the analysis of bone carbonate to geographical locations using established $\delta^{18}O$ maps of tap water for the UK.

This study utilised a small number of samples, and it is acknowledged that this is only preliminary research. It is essential that a greater number of individuals are sampled, both for bone and hair material, in order draw more accurate and meaningful conclusions from the data.

Acknowledgements

Firstly I would like to thank **Dr. Wolfram Meier-Augenstein** for his development of the method, without which this research could not have taken place. The experience and knowledge of **Dr. Helen Kemp** has been crucial during the sample analysis stage of this study. **Professor Sue Black, Professor David Hopkins and Dr. Derek Stewart** have all dedicated a considerable amount of time making recommendations as to the content and structure of this thesis, for which I am truly grateful. I would also like to thank both **Dr. Charlie Scrimegeour** and **Dr. Susie Fawley** for their explanations of concepts relating to stable isotope analysis, and advising on sections of this thesis. Finally I am grateful to **Netta Gallazzi** for her assistance in the collection of bone samples used in this research.

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DNA	Deoxyribonucleic acid
NDNAD	National DNA Database
NPIA	National Policing Improvement Agency
IRMS	Isotope ratio mass spectrometer
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water
VCDT	Vienna Canyon Diablo Trolite
AIR	Atmospheric air
IAEA	International Atomic Energy Agency
SIP	Stable isotope profiling
WMO	World Meteorological Organisation
GNIP	Global Network of Isotopes in Precipitation
GIS	Geographic information systems
CAM	Crassulacean acid metabolism
Trux	Truxilline
TMC	Trimethoxycocaine
ANOVA	Analysis of variance

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Chapter 1: Introduction

1.1 Introduction

The identification of individuals has been an important aspect of society for thousands of years with biological characteristics such as gait, the voice, fingerprints, and facial features being used for recognition. For example, ancient Egyptians identified traders based on features such as height and eye colour (Ashbourn, 1994), and potters from East Asia used fingerprints as a method of brand identity (Toth, 2005). The first application of biological characteristics in the criminal justice system began in the mid-19th century with simple body dimensions recorded by Alphonse Bertillon (Cascetta and De Luccia, 2004; Jain *et al.*, 2004a). Towards the end of the 19th century Bertillon measurements were eventually made redundant in favour of fingerprints which were considered a more distinctive and practical method (Cascetta and De Luccia, 2004; Jain *et al.*, 2004b). Human identification techniques have since developed considerably, drawing on a number of different subject areas including archaeology, medicine, chemistry, biology and geology. The past 20 years in particular has seen an increase in the development of more sophisticated methods including trace element analysis and DNA typing over more conventional ones such as morphometrics. Television programmes such as 'CSI' and 'Bones' have glamourised the roles of forensic scientists, and immortalised the techniques they employ. In reality a greater degree of sophistication often translates into increased costs of analysis, as with DNA typing (Corach *et al.*, 2005). It may be that certain methods are inapplicable in particular situations (as with highly degraded remains, where it can be difficult to extract a useful DNA profile) (Rutty *et al.*, 2005). It must also be acknowledged that even if a profile can be obtained, there may not be a suitable profile for comparison, rendering the technique useless for identification of remains. Despite the retention of nearly 5 million individual profiles on the UK NDNAD (National DNA Database) (correct as of 31/12/2009; (NPIA, 2010)), the information is of very little use in cases of missing persons or mass disasters. The issue results from the fact that many of the entries on the database are from convicted criminals, and do not represent a large proportion of the population.

Forensic investigations of both living and deceased individuals may require the expertise of an anthropologist. One of their roles may be to create a biological profile by assessing the age, sex, ancestry and stature of an individual. An anthropologist may also provide information on any pathological conditions and trauma evident on the remains. However this alone may not be enough to provide investigators with a definitive identification, as often a substantial amount of the population could be identified using the description offered by the forensic anthropologist. For example, human skeletal remains could suggest the individual is female, Caucasoid, between the ages of twenty and thirty years, and between 5'4" and 5'7" in stature. This describes a significant proportion of the UK population, and is likely to be of little value for identification purposes (although it does substantially decrease the search parameter).

Interpol specify three main types of identifiers; primary, secondary and accessory. Primary identifiers are considered unique, and can be applied on their own to confirm the identity of an individual (Thompson and Puxley, 2007). Primary criteria include odontology, DNA, fingerprints, and unique medical conditions (for example an implant with a serial number). Secondary identifiers include distinctive scars, blood group and personal effects, with at least two secondary criteria required for identification. It is understood that accessory identifiers will not be accepted unless via exclusion in a closed incident (where the numbers and identities of victims are more readily known, for example an incident involving an airplane), and should be utilised in conjunction with other criteria (Thompson and Puxley, 2007) (see Table 1).

Table 1: Criteria applied when identifying the deceased

Criteria	Examples
Primary	Odontology DNA Fingerprints Unique medical conditions
Secondary	Personal effects Blood group X-rays Distinctive scars Distinctive marks Jewellery Physical disease Body modifications
Accessory	Visual identification Clothing Photographs Description Body location

Producing a basic biological profile can become extremely challenging when human remains are in a particularly poor condition, for example highly fragmented (as with the remains from the World Trade Centre), or those that have been exposed to destructive environmental and/or taphonomic processes for a prolonged period of time (as with the Asian tsunami). DNA typing was of restricted value during the identification of remains from the Asian tsunami due to a high level of DNA degradation, and the expense and high time consumption of the process. Practitioners relied heavily on other primary criteria such as odontological and fingerprint evidence, however if adequate ante-mortem data was not available (for example in countries with poor dental practices) then these techniques were also of little use.

Another issue complicating identification of unknown remains is the world-wide travel possibilities available. It is now relatively easy for any individual to travel, or perhaps even relocate to a different geographical location. This ease of movement does not allow the assumption that may previously have been made, that remains are likely to be from the local area. The example of the Asian tsunami where thousands of deceased individuals were of European origin, illustrates this point (Abbasi, 2005). Identification

of living individuals is also more challenging, with illegal immigrants and criminals providing fake documentation and information to authorities with the view to entering and subsequently residing in a certain country (Sadiq, 2005). It is possible terrorists for example, may travel undetected to a variety of countries where they are able to access training in for example, bomb production and weapons handling (Nesser, 2008). It is difficult to track and monitor the movements of these individuals, particularly if they travel on false documentation or under assumed identities. The forensic anthropologist has therefore, a challenging job which is only likely to increase in complexity as geographical relocation becomes more affordable and straightforward.

In light of these issues, it seems logical to research and develop forensic techniques designed to overcome the complications associated with traditional morphological and metric procedures. It is essential for the forensic anthropologist to move beyond the conventional biological profile, and employ more modern methods that are able to reveal supplementary information about an individual. Additional indications of identity such as geographical origin and recent movement could prove invaluable in forensic investigations, particularly those where little evidence is available.

A relatively novel technique in the field of human identification is the use of stable isotopes. The use of stable isotopes in scientific studies has its roots in subjects such as biology (Plentl and Schoenheimer, 1944; Rittenberg and Foster, 1940) and geochemistry (Ault and Kulp, 1959; Craig, 1953), and is widely used in archaeological research (Hedges and Reynard, 2007; Macko *et al.*, 1999). Isotopes have been traditionally used in human research in medicine (for drug, and nutritional studies) (Matwiyoff, 1973), archaeology (Cerling *et al.*, 1997; DeNiro, 1985; Sealy and Van Der Merwe, 1985), and paleoanthropology etc (Bocherens *et al.*, 2007; DeNiro, 1985; Longinelli, 1984). Very little research has been conducted into the use of elemental isotope analysis for human identification. Stable isotopes have often been used to reconstruct diet and movements of ancient populations, but it is only recently that this technique has been applied in the field of forensic science (Benson *et al.*, 2006; Pye and Croft, 2004). Much of the research in stable isotopes focuses on wildlife, in particular the migratory patterns of birds and

butterflies (Hobson, 2005; Hobson *et al.*, 1999b; Wassenaar and Hobson, 1998), and the dietary intake of mammals (Cerling and Harris, 1999; Walker *et al.*, 1999). Medical and archaeological studies have utilised the isotopic content of a number of human tissues including teeth, bone, nail, and hair to draw conclusions (DeNiro, 1985; Fuller *et al.*, 2006; Fuller *et al.*, 2004; Nakahara *et al.*, 1992; Wright and Schwarcz, 1998). This research, despite demonstrating the same basic isotopic principles, should not be used for comparison purposes with data collected from modern-day humans. The large difference in metabolic rates between humans and animals such as birds equates to substantial variation in tissue turnover (Kohn, 1996), and thus isotopic uptake. In addition differences in the isotope content of body tissues may result from the 'global' diet consumed by modern humans.

Despite the lack of modern-human isotopic data available, stable isotope profiling is being used in forensic scenarios involving human identification, and has even been used in murder investigations (Meier-Augenstein and Fraser, 2008). A large proportion of casework utilising isotopic analysis is often related to drugs, and attempting to link samples back to a larger batch or geographical region (Ehleringer *et al.*, 1999). The use of human tissue samples for forensic stable isotope analysis is relatively rare, with few case studies (for example 'The Torso in the Thames' (O'Reilly, 2007)) and little research available, but the potentials are extensive. This technique could overcome some of the issues associated with modern methods. Stable isotope analysis can be performed on highly fragmented and damaged remains, and at a relatively low cost in comparison with (for example) DNA typing. Although it is highly unlikely that isotopic signatures will be able to provide enough evidence to be considered a primary or even secondary identifier, they may be capable of giving investigators clues as to an individual's provenance, recent geographical movements, and dietary intake (Meier-Augenstein and Fraser, 2008; O'Connell *et al.*, 2001). This information may narrow the search criteria, focus resources, and has the potential to assist towards the establishment of a positive identification. Research in the area of stable isotopes in human identification is on the increase, but there is still a substantial amount of basic information that is required before identification using this technique can be given a high level of credibility. Isotopic

techniques have yet to be scrutinised in a courtroom environment, with topics such as inter- and intra- subject variability unexplored. This is a current limitation, as information would be used to determine the probative value of the evidence produced. For example, judges could evaluate the errors and variability of a method before determining whether to declare complex scientific evidence admissible in Court, and barristers use the figures to either support or undermine the credibility of evidence proffered. It is therefore crucial that this area of profiling is explored further and reliable, quantifiable results are produced that can be of probative value in the judicial system. The research presented in this thesis will attempt to address these issues by describing bone and hair data collected from several individuals in studies designed to establish inter- and intra- individual variability for use in forensic scenarios.

Chapter 2: Elements and Isotopes

2.1 Introduction

The Earth and its atmosphere are comprised of over 90 known elements. The smallest unit of an element, the atom, is composed of protons, electrons, and neutrons. There are essentially two parts to an atom, a nucleus and orbitals (electron pathways). The protons and neutrons reside within the nucleus, while the electrons orbit the nucleus. The number of protons and electrons are always equal in elemental atoms; it is the number of neutrons that may differ. Of the more than 90 naturally present elements comprising the Earth and its atmosphere, around two thirds occur in more than one form, each with a varying number of neutrons, called isotopes. This chapter will discuss isotopes in detail, the differences between radioactive and stable, variation in reaction speeds, and the number of isotopes associated with certain elements. It will also provide information on the standards used in the measurement of isotopes, and a brief description of two important processes; fractionation and mixing.

2.2 Overview of Stable Isotopes

The term 'isotope' was coined by Margret Todd (a Scottish physician) in 1913, and first used by Frederick Soddy (an English radiochemist and winner of the Nobel Prize in 1921). The word 'isotope' is derived from the Greek *isos* meaning equal, and *tópos* meaning position or place (Fry, 2008). It refers to the periodic table (see Figure 1), and the fact that all isotopes of the same element occupy an identical site on the periodic table. Chemically speaking, all isotopes react in the same way, as this is largely governed by electronic configuration (Hoefs, 2009). Variation in the number of neutrons does however mean that isotopes of the same element will have different atomic masses (the sum of the number of protons and neutrons). It is understood that if atoms of elements are present in a variety of isotopic forms during a chemical reaction, then it is likely that there will be an uneven distribution of these isotopes between the products and reactants (Urey, 1947). This process is called isotopic fractionation and will be discussed in greater detail later in this chapter.

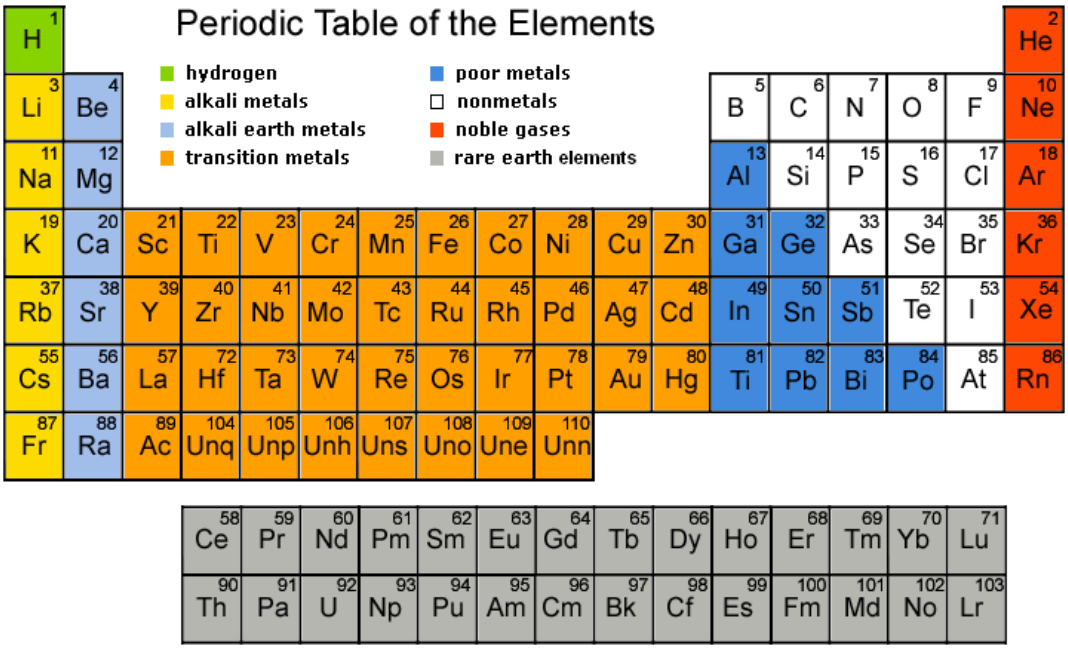

Figure 1. An image of the periodic table (Artbranch, 2010).

Isotopes can be divided into two fundamental kinds, stable and unstable (radioactive species). The number of stable isotopes is around 300 whilst over 1200 unstable isotopes have been discovered so far. Stable isotopes are those that do not undergo radioactive decay, and thus have nuclear stability and their masses remain the same. Radioactive isotopes (also called radioisotopes) have unstable nuclei that undergo decay and emit radioactive rays which can be in the form of electromagnetic radiation (gamma radiation), or discrete particles such as electrons (beta radiation) or He nuclei (alpha radiation). The rate of decay of some radioisotopes has been extensively studied, such as that of ^{14}C. Decay of radiocarbon has been utilised for many years as a technique for dating organic materials (Bronk Ramsey, 2008; Hedges and Van Klinken, 1992; Longinelli, 1984). Stable isotopes (given that they do not alter over time) have also been used extensively in research, they have a vast array of applications from medicine to cosmology.

There are two main elements and their stable isotopes that are of interest in this research (oxygen and carbon). Carbon has two stable isotopes ^{12}C and ^{13}C and oxygen has three

isotopes O^{16}, O^{17} and O^{18}, all of which are stable. The lightest of an element's isotopes (12 for carbon, and 16 for oxygen) are generally the most abundant in the environment (see Table 2), and are more readily influenced by biological and physical processes than the heavier isotopes (Bell, 2006).

Table 2: Elements, their stable isotopes, and natural abundances

Element	Stable Isotope	Abundance (%)
C	**^{12}C**	**99.89**
	^{13}C	**1.11**
O	**^{16}O**	**99.76**
	^{17}O	**0.04**
	^{18}O	**0.20**

2.3 Measurement and Instrumentation

Isotopic analysis is typically performed using a specialist type of instrumentation called an isotope ratio mass spectrometer (IRMS). This is a highly sensitive and specialised form of mass spectrometer capable of instrumental precision of <0.02‰ (see page 10 for ‰ definition) and standard deviation of <±0.01‰ (Hoefs, 2009). As the name suggests, a mass spectrometer measures the masses of the elements comprising a sample. Samples are introduced into the mass spectrometer and the following processes occur;

1. samples undergo ionisation via electron bombardment
2. ions are accelerated through a magnetic field
3. the separation of ions of different masses takes place on the basis of their mass/charge ratio
4. resolved beams are collected simultaneously in a series of faraday cups
5. the current created when the beams strike the faraday cups is used to compute the stable isotope ratios

Previously, solid materials undergoing mass spectrometric analysis required conversion into a gaseous form isotopically representative of the original sample prior to entering the mass spectrometer. This meant the manual conversion and injection into the instrument, as automated introduction mechanisms were non-existent (Benson *et al*., 2006). These

steps were time consuming and increased expense and the possibility of contamination (Meier-Augenstein and Liu, 2004). Modern mass spectrometry equipment can be combined with a variety of perhipheral devices, making the gaseous conversion and injection processes fully automated. This development in technology has eliminated external manipulation, minimising both expense and instances of contamination (Meier-Augenstein and Liu, 2004). There are several types of isotopic analysis, with the majority fitting into two general categories; compound-specific and bulk analysis. Compound specific analysis separates the constituent compounds within a complex sample and can provide an isotope value for each individual compound (Carter *et al*., 2005).

Isotope values are generally reported as 'delta' values (δ), and are ratios that compare the isotopic composition of a standard. The original international standard for carbon was taken from a *Belemnitella americana* fossil, originating from the Peedee formation in South Carolina. Peedee Belemnite has been assigned the value of 0 (Ambrose and Norr, 1993; Van Der Merwe *et al.*, 2003). The material used to produce this standard has since been completely exhausted and a new standard called V-PDB (or Vienna-Peedee belemnite) has been produced. This standard has a value extremely close to that of the original. Other standards include Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen, and Vienna Canyon Diablo Trolite (VCDT) for sulphur (Hoefs, 2009). Nitrogen ($\delta^{15}N$) values are expressed relative to AIR (atmospheric nitrogen) standard, and are generally positive (Ambrose and Norr, 1993). These standards are routinely used to calculate δ values of unknown samples and are preferred over other existing standards as they imply that the measurements have been calibrated in accordance with the International Atomic Energy Agency (IAEA) guidelines.

The result of isotopic analysis is expressed using the following equation. The delta value is expressed in parts per thousand (‰) difference (δ) compared to a standard. This is calculated for (as an example) oxygen as:

$$\delta^{18}O = \frac{^{18}O/^{16}O \text{ (sample)} - ^{18}O/^{16}O \text{ (standard)}}{^{18}O/^{16}O \text{ (standard)}} \times 1000 \quad (‰)$$

Delta values are reported as either higher (enriched in the heavier isotope) or lower (depleted in the heavier isotope) when compared with a standard (Kendall and Coplen, 2001). For example, if a sample is calculated to have a delta value of +10‰ then it is ten parts in 1000 enriched in ^{18}O when compared with the standard. If the delta value is -10‰ $\delta^{18}O$ then it is ten parts in 1000 depleted in ^{18}O. The resulting value is multiplied by 1000 as the difference in abundance of two isotopes is often small, with the measured variation beginning in the second or third decimal digit (Schoeller, 1999).

2.4 The Principles of Isotope Fractionation

Abundances of elemental isotopes vary, and constantly undergo partitioning in a process called fractionation (Hoefs, 2009). The variations in physical and chemical properties of isotopic compounds (molecules containing different isotopes of the same element) are brought about by variation in the mass of the nuclei. The variation in mass results in molecules containing the heavier isotope (or heavier isotopes), having firstly, a lower mobility, and secondly, being able to form stronger bonds (Mook and de Vries, 2000). As a consequence of lower mobility, molecules containing heavier isotopes will have a lower diffusion velocity. Molecules containing heavier isotopes will also have a lower collision frequency (meaning they react more slowly in comparison with molecules containing the lighter isotope). The ability of heavier isotopes to form stronger bonds with other atoms means that more energy is required to break the bond between an atom bonded to a heavy isotope of a given element relative to an atom being bonded to the lighter isotope of the same element. For example, more energy is required to break the bond between ^{1}H and ^{2}H (or deuterium (D)) than is required to break the bond between two atoms of ^{1}H. Similarly, more energy is required to break the bonding between two deuterium atoms than between H and D (see Figure 2) (Hoefs, 2009; Mook and de Vries, 2000). If a reaction does not go to completion, the consequences of mass variation result in the product containing more of the light isotope and less of the heavy isotope (Parkes, 1986); If the reaction does complete, the cumulative product will have the same isotopic composition as the original substrate (Sulzman, 2007).

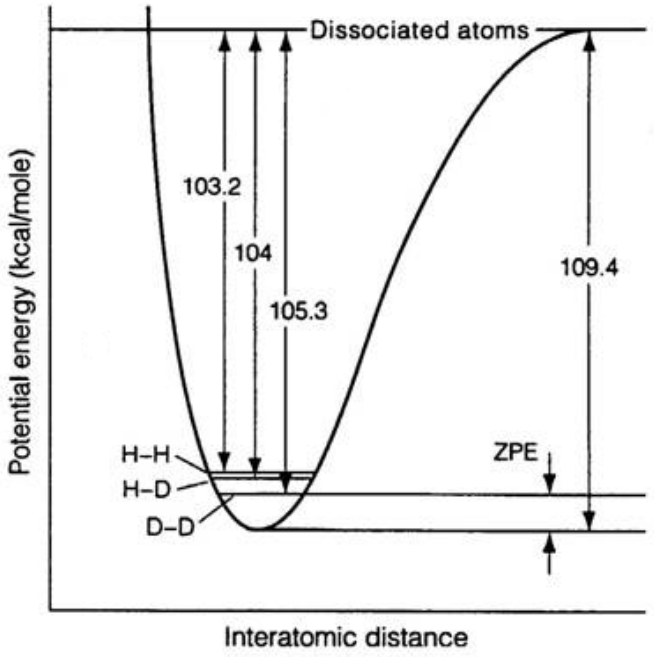

Figure 2: (O'Neil, 1986) The potential energy relationship for stable hydrogen isotopes of a molecule. As can be seen, a greater amount of potential energy (105.3kcal/mole) is required to break the bond between two ^{2}H isotopes, than between two ^{1}H isotopes (103.2kcal/mole). ZPE (zero point energy) is defined as the kinetic energy retained by molecules within a substance at a temperature of absolute zero (McGraw-Hill, 2003).

2.4.1 Fractionation in Living Organisms

Isotopic fractionation within organisms is a result of both extraneous and intrinsic influences (Hedges and Reynard, 2007; Londry and Des Marais, 2003; Post, 2002). Extraneous influences altering isotopic ratios include geographic and climatic conditions such as temperature, humidity, continentality (distance from the sea) and altitude (Dansgaard, 1964). The isotopic equilibration of organisms with their environment means the tissues of plants and animals in a particular area will isotopically reflect the region (Renou *et al*., 2004; Wunder *et al*., 2005).

Intrinsic physiological factors may also influence isotopic ratios in living tissues. Studies have demonstrated that diet, body size, metabolism and heat loss may result in significant variation in the level of fractionation in certain species (Kohn, 1996; Kohn *et al*., 1996). It is therefore essential for researchers to consider the physiological adaptations and

dietary habits of the species under observation. It has also been well documented that bone remodelling rates (see Chapter 5) affect isotopic compositions. This is a result of the variation in bone turnover rates between different tissue types and skeletal elements (Chamay, 1972; Pate, 1994). It is therefore important in isotopic research of skeletal elements to sample consistently the same bone from each individual.

Chapter 3: An Overview of Isotopes in Research

3.1 Introduction

This chapter focuses on literature detailing the use of stable isotopes. Stables isotopes have been applied in scientific studies for many years, and in a variety of fields. This has led to an abundance of data available to researchers wishing to employ techniques associated with stable isotope analysis to their own experiments. Despite this large volume of work, relatively little work has been conducted on the use of stable isotope for human identification.

3.2 Stable Isotopes in Research

The use of stable isotopes in research began with studies in biology, with techniques used to label dietary molecules in rats, and establish their uptake into the body (Foster *et al.*, 1939; Plentl and Schoenheimer, 1944; Schoenheimer *et al.*, 1938). The basic techniques applied in these studies, and the understanding of isotopes and their potential in research has developed substantially over the years. Much of the recent research involving stable isotopes has been from a hydrologic and an ecological perspective, and has focused on tracing water through the hydrologic cycle (Gat and Issar, 1974; Worden *et al.*, 2007; Yamanaka *et al.*, 2007), establishing food webs (Corbisier *et al.*, 2006; Schmidt *et al.*, 2007) , determining photosynthetic pathways in plants (Cousins *et al.*, 2007; Tipple and Pagani, 2007), tracking the migratory patterns of birds (Hobson, 2005; Wunder *et al.*, 2005) and butterflies (Brattström *et al.*, 2008; Miller *et al.*, 2011), and monitoring the dietary intake of mammals (Hobson *et al.*, 1999a; Iacumin *et al.*, 2005; Sponheimer *et al.*, 2003). More current applications include isotopic investigations of food adulteration, explosive materials, and illicit drugs (Meier-Augenstein, 2010).

Studies involving human subjects have utilised a wide variety of sample materials (O'Connell *et al.*, 2001). These investigations have often had their foundations in medicine (Koletzko *et al.*, 1998) and archaeology (Hoppe *et al.*, 2003; Macko *et al.*, 1999). Pioneering research by archaeologists Van der Merwe and Vogel (1978) illustrated the potential of isotopes for revealing dietary information from human tissue.

Their work on the introduction of maize to the North American diet by analysing bone collagen encouraged further isotopic study using human tissues, with archaeologists developing techniques to analyse tooth, nail and hair samples (Van Der Merwe and Vogel, 1978; White, 1993; Wright and Schwarcz, 1998; Yoshinaga *et al.*, 1996). This archaeological research also attracted the attention of forensic scientists who realised isotopic analysis may be applied to casework involving identification issues.

The use of stable isotopes in forensic science has increased substantially over the past 20 years, particularly with the development of new analytical techniques and increasingly sophisticated and precise analytical technology. Forensic scientists have used the same basic isotopic principles discovered through archaeological and ecological research, and adapted the analytical techniques to conduct isotopic research on materials that may be encountered in casework. These materials have included wooden safety matches (Farmer *et al*., 2005), architectural paint (Reidy *et al.*, 2005), drug-based samples (Ehleringer *et al*., 1999), explosive materials (Ader *et al*., 2001), and adulterated foodstuffs (Padovan *et al*., 2003). Stable isotope signatures are a particularly useful tool for forensic investigators. They are able to provide information suggestive of the geographical, biological and/or chemical origin of the material under observation. They are also capable of distinguishing between two seemingly 'identical' materials (Meier-Augenstein, 2007). In essence they are an invaluable means of providing comparative analysis of materials of interest in forensic casework.

3.3 Stable Isotopes in Human Identification

3.3.1 Principles of Stable Isotope Profiling

In recent times, headlines have been dominated by mass disaster incidents such as the London tube bombings (2005) and the Asian tsunami (2004). It can therefore be argued that human identification and its methods and techniques have never been under greater scrutiny than today. The development of new procedures in this field is essential to overcome problems associated with the employment of traditional techniques, including fingerprint analysis (for example decomposition of soft tissue) and DNA profiling (such as no reference profile for comparison). One such method currently under investigation is stable isotope profiling (SIP). SIP is able to exploit the relationships between isotopic

content of an individual's diet, the isotopic composition of their body tissue, and geo-location or recent travels. In simple terms, this method has the potential to map the same aspects of an individual's past through isotopic analysis of their body tissues. This type of information is of particular use as it allows investigators to focus their resources for example, by excluding particular countries and/or regions from their search (Meier-Augenstein and Fraser, 2008).

The fundamental principle in establishing geographic history and lifestyle using SIP is that an individual's only source of carbon and nitrogen is in their dietary intake. In a similar fashion, an individual's major source of hydrogen is from water (H_2O) consumed through their diet, either as liquid or as part of fruit or vegetables. Since all drinking water is ultimately derived from snow and rainfall, processes such as evaporation, condensation, and precipitation are reflected in the isotopic composition of drinking water (Darling *et al.*, 2003; Darling and Talbot, 2003). Mass discrimination during these processes causes meteoric water (precipitation), and subsequently drinking water, to vary in isotopic composition depending upon geo-location. Accurate maps detailing the global distribution of water isotopes are available and allow investigators to link the isotopic composition of water samples to a particular geographic location and/or region (Ehleringer *et al*., 2008).

It has been established that variations in the isotopic abundance of light elements in compounds constructing human tissues (hair, nails, bones, teeth) reflect the isotopic constituents of food and drink consumed during their formation (Fraser and Meier-Augenstein, 2007; Fraser *et al.*, 2006; Nardoto *et al.*, 2006; Sharp *et al.*, 2003), and the isotopic signature of these ingested materials can in turn be linked to geographical locations (Ehleringer *et al*., 2008). The well documented variation in growth rate of human tissues means that isotopic analysis of several tissues can allow investigators to construct a chronology of events and document geographical movement (Wilson and Gilbert, 2007). Isotopic signatures of hair and nail samples can be indicative (depending upon length) of recent travels i.e. those occurring weeks or months previously. This is due to the constant and relatively rapid construction of these tissues. In a similar fashion

bone is constantly remodelling, however this regeneration is considerably slower than the growth associated with hair and nail. Teeth can be analysed to reveal the geographical location of an individual when the tooth was formed (i.e. childhood/adolescence), as once deposited in the enamel, the elemental isotopic ratios do not alter during a person's lifetime (Wright and Schwarcz, 1998).

It is the strong relationship between geographic location, the isotopic signature of dietary intake, and the isotopic content of body tissues which makes stable isotope profiling an invaluable tool for forensic scientists. These links allow forensic investigators to establish the provenance of an individual and/or chronological timescales for their geographical movement. Although this information is dependent upon the type(s) of tissue available, the data resulting from stable isotope profiling can used in a number of scenarios which are detailed in the following chapter.

3.3.2 Stable Isotopes as an Identification Technique

Stable isotope profiling is of particular use when attempting to gain information from remains that yield few clues with regard to identification. SIP could have been particularly useful during the processing of thousands of victims of the Asian tsunami (2004). Viable DNA could not be extracted from many individuals as remains were in the latter stages of decomposition, and even if viable DNA could be extracted or fingerprints taken, there was no guarantee of a match in any database. These identification techniques were also costly, and analysis a lengthy process, particularly with the number of individuals involved (over 200,000). In simple terms, there were no reliable means of distinguishing visitors to the area from indigenous individuals. This led to victims being identified visually using facial characteristics typically associated with the local population. The remains of some of these victims were released to local families and allowed to be buried. Concerns were raised by foreign forensic teams who appreciated that many countries, such as the UK, have nationals from a variety of ethnic groups (UKGOV). Consequently, some foreign nationals may have facial characteristics similar to that of the indigenous population. As a result, international forensic teams persuaded local authorities to permit exhumation and re-analyse victims previously identified by

visual means. It was recognised that for a disaster of this magnitude methods such as DNA analysis and fingerprinting were too refined. This scenario required a method capable of separating victims into broad categorisations rapidly, i.e. distinguishing between visiting individuals and the indigenous population. Stable isotope profiling would have been of great benefit in the identification process after the tsunami. When compared with more traditional approaches it is rapid and cost effective, with the subsequent data able to indicate an individual's provenance or recent life history. This information may suggest the victim's nationality and lead to rapid and accurate identification, and thus repatriation.

Stable isotope profiling is not limited to mass disaster scenarios, but may also be applied to the living, for example in situations where ascertaining the recent geographical history of a person is vital. This could be in relation to people and/or drug smuggling (Fraser *et al*., 2006), terrorism, or murder investigations (Fraser and Meier-Augenstein, 2007). The recent movements of an individual may assist police in verifying or disproving statements, and lead investigators to possible identities for people with no known history or those that are unwilling to cooperate with officials. Stable isotope profiling also has an important role to play in cold cases where detectives may require additional leads to reopen the investigation. Although unable to provide outright identification, the data generated by analysing the isotopic content of body tissues can contribute information suggesting dietary intake and geolocation (Cerling *et al*., 2003; Ehleringer *et al*., 2008; Meier-Augenstein and Fraser, 2008; Sharp *et al*., 2003). It may also indicate lifestyle choices such as a high protein diet, or if an individual was a vegetarian or vegan (O'Connell and Hedges, 1999). In essence stable isotope profiling has the potential to assist forensic investigations by providing new leads, focusing resources, as well as greatly reducing the potential identities for an individual (Meier-Augenstein, 2010).

The major issue surrounding the use of stable isotope profiling is the lack of associated data. As with many other identification methods it requires comparison of unknown samples with one or more references. The current inadequacy of comparative databases is hampering the application of stable isotope profiling as an identification technique. Many

of the isotopic reference profiles are sourced from plant or animal material, or ancient human populations. The values obtained from these studies may not be representative of contemporary human tissues and are therefore may be of little value in forensic casework. The generation of appropriate reference models is essential for comparison of modern human bone, hair, teeth and nail, with both national (reflecting the isotopic signatures within a nation) and international (detailing global signatures) databases required.

Chapter 4: Isotopes and the Environment

4.1 Introduction

This chapter will focus on water- and plant-based isotopic research. It will discuss the movement of ^{18}O and ^{2}H isotopes in the hydrologic cycle, outline the processes involved, and their effects on isotopic composition. The chapter will also detail the geographical variation in isotopic signatures, with reference to seasonal and environmental patterns. A short section will provide a summary of research utilised to produce global (and regional) ^{18}O and ^{2}H precipitation maps, and how these can be used when attempting to link an individual to a specific geographical area. The chapter will conclude with a discussion of isotopes in plants, with particular reference to the variation in their isotopic signatures, and the environmental factors affecting these. This section will also provide a brief description of how the isotopic signatures from plant-based material can be used in forensic casework, focussing mainly on human identification.

4.2 The Hydrologic Cycle

Water is an essential part of the environment with many organisms depending upon water for nutrition, and biosynthetic processes such as those during metabolism. Approximately 95% of water on Earth is found within the oceans, and is transported through the hydrosphere (the region containing the combined mass of water under, over, and on the surface of the planet) via a collection of processes called the hydrologic cycle (Gat, 1996).

The hydrologic cycle (an overview of which can be seen in Figure 3) commences with the evaporation (the process by which water changes phase from liquid to vapour) of water from ocean surfaces. The majority of evaporation occurs from oceans, with the remaining occurring from inland water and vegetation (Dansgaard, 1964). As this moisture is lifted, it cools and condenses to form clouds. Wind transports clouds around the globe until the moisture contained within, falls as precipitation. There are several forms of precipitation; sleet, snow, hail, with the most common for the UK being rain. Once on the Earth's surface, one of two processes may occur (Gat, 1996); evaporation of water back into the atmosphere, or it may permeate the Earth's surface to become

groundwater. Groundwater may either then seep into streams, rivers, oceans, or is released back into the atmosphere via transpiration. Transpiration is the process by which water returns to the atmosphere via evaporation from the leaves and stems of plants. Precipitation that does not evaporate, transpire, or penetrate the ground is called runoff, and empties into lakes, streams, rivers, and finally oceans where the hydrologic cycle can begin again (Gat, 1996).

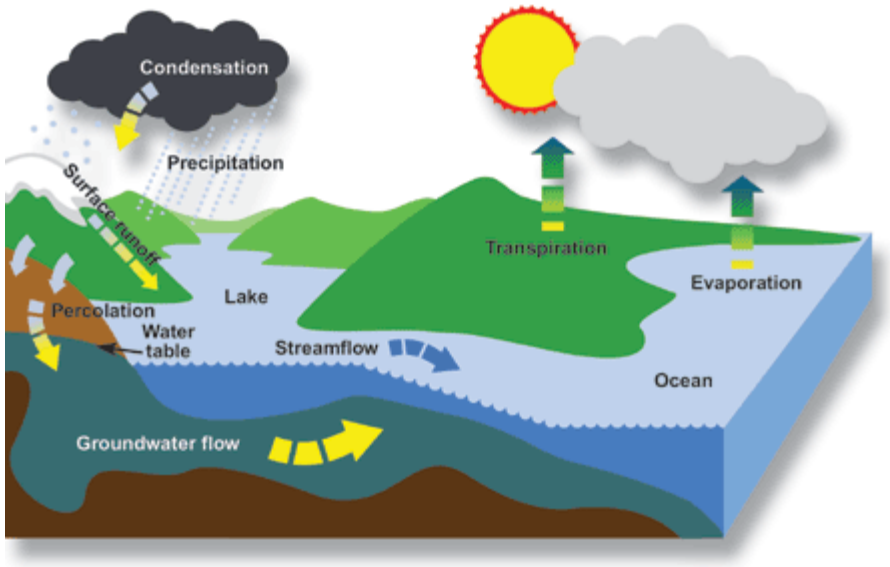

Figure 3. (DAWN, 2008) The Hydrologic Cycle. This image demonstrates the various processes that form the hydrologic cycle and contribute to the movement of water across the globe.

As water moves through the hydrologic cycle, it undergoes mass-dependent fractionation (see Chapter 2 for an explanation), in which ^{1}H and ^{16}O (the lighter isotopes) evaporate more readily than heavier isotopes (^{2}H, ^{17}O and ^{18}O). This leads to depletion of meteoric waters (i.e. atmospheric moisture, precipitation, and the ground and surface waters derived from them) when compared with ocean waters, with regards to the lighter isotopes (i.e. a more negative δ value). In condensation reactions, the heavier isotopes are favoured meaning precipitation falling from a cloud will have a more positive δ value (i.e. be enriched in heavy isotopes) than the cloud vapour from which it was formed. As evaporation from the ocean occurs, isotopic fractionation favours the light isotopes meaning the δ values for the meteoric waters are often negative when compared with VSMOW (assigned the value of 0‰) (Craig, 1961).

Evaporation and condensation reactions occur constantly during the process of cloud production and precipitation formation. These reactions occur both over the oceans, and as a result of wind transportation of clouds, over continents. Each process in the hydrologic cycle causes a slight variation in the isotopic composition of water, meaning the more evaporation and condensation reactions occurring as meteorological waters are transported inland, the more negative the δ value of precipitation (see Figure 4). The condensation of water vapour in cloud favours heavier isotopes, and therefore precipitation has a less negative δ value than the moisture within the cloud (see Figure 4).

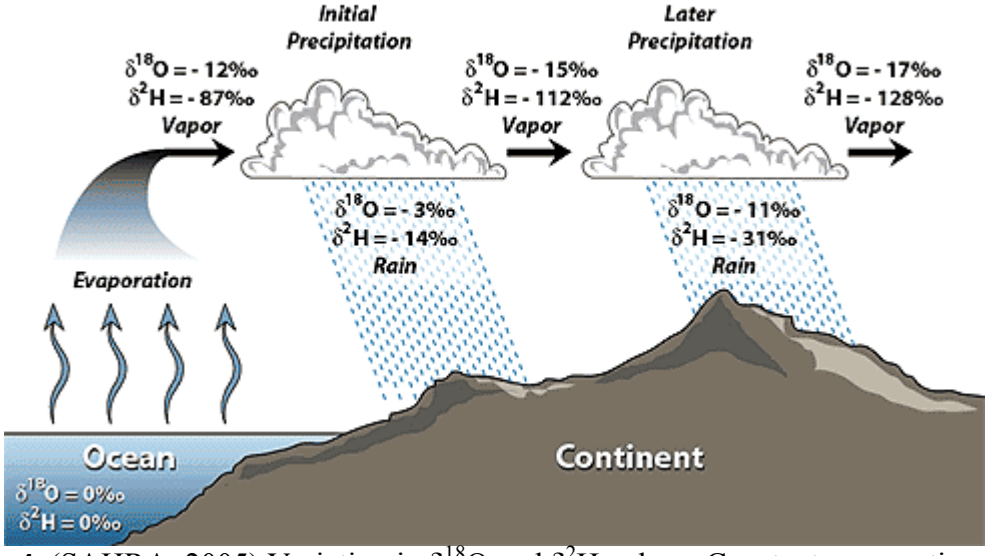

Figure 4. (SAHRA, 2005) Variation in $\delta^{18}O$ and $\delta^{2}H$ values. Constant evaporation and condensation reactions cause isotopic fractionation, and therefore the δ values of ^{18}O and ^{2}H in precipitation to vary across the globe.

Since the discovery of the heavy isotopes of hydrogen and oxygen, scientists have been able to observe significant variation in the isotopic contents of ocean water, freshwater, and snow (Darling *et al.*, 2003; Darling and Talbot, 2003). The development of more sophisticated measurement techniques has led to increased observation of climatic factors influencing the isotopic composition of precipitation such as amount of rain, surface air temperature, and the altitude and latitude (Aggarwal *et al.*, 2010). The application of isotopes to quantify transitions in the hydrologic cycle had been established by the 1950s, but these were fairly primitive with limited scope and records of measurement (Craig,

1961; Dansgaard, 1964). It was the result of nuclear testing in the 1950s and the subsequent requirements to monitor tritium (^3H) levels that led to a substantial increase in the number and geographic location of stable isotope measurements in the hydrologic cycle (Dansgaard, 1964).

4.3 Geographical Variation in Isotopic Signatures

The geographical variation of isotopic signatures resulting from fractionation processes in the hydrologic cycle follows predictable patterns. Dansgaard (1964) analysed a substantial amount of data from global distributions of stable isotopes, and subsequently identified several factors resulting in variation of isotopic values. Dansgaard listed a number of physical and meteorological determinants such as altitude, latitude, distance from the coast, surface air temperature, and amount of precipitation. These so called 'effects' were substantiated by other studies (Bowen and Wilkinson, 2002; Gat, 1996; Ingraham, 1998), and were deemed to be a result of isotope fractionation associated with phase changes of water in the hydrologic cycle. Some of the variation is a result of isotope fractionation when atmospheric water vapour is condensed to produce precipitation. The air masses lose water as they proceed from lower to higher elevations (also called the 'altitude effect'), along temperature gradients from tropical to polar latitudes (the 'latitudinal effect') and the coast to inland (the 'continental effect') (Gourcy *et al*., 2005). It has also been established that seasonal and inter-annual patterns exist in the isotopic content of precipitation (Aggarwal *et al*., 2010). Figure 5 illustrates the latitudinal effect, continental effect and altitude effect on the average δ^2H values of meteoric water in North America. The values become more negative (i.e. more light isotope) with increasing latitude and towards the continental interior. They also demonstrate sharp variation in mountainous regions, most notably around the Sierra Nevada range in California (black circle Figure 5).

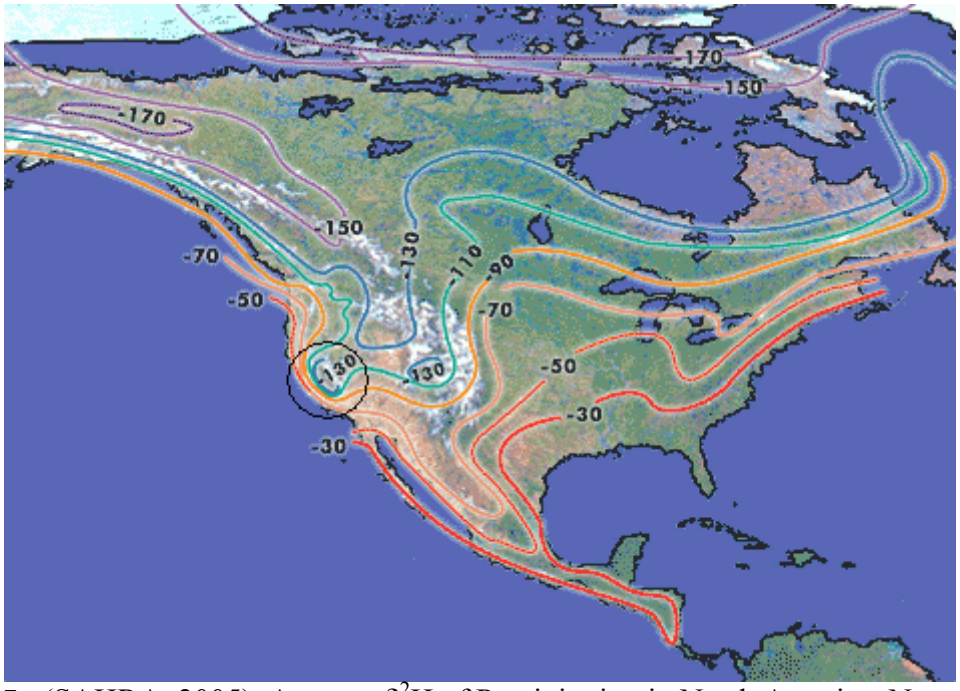

Figure 5 . (SAHRA, 2005). Average δ^2H of Precipitation in North America. Note the latitudinal, continental, and altitudinal effects.

The International Atomic Energy Agency's (IAEA) Water Resources Programme and the World Meteorological Organisation (WMO) have been monitoring and recording the levels of hydrogen and oxygen isotopes in precipitation since 1961 (Aggarwal *et al.*, 2010). The primary objective was the collection of data based on isotopic content of precipitation on a global level. There are currently 183 stations contributing daily and/or monthly samples from 53 countries to the GNIP (Global Network of Isotopes in Precipitation) database (the locations of stations can be seen in Figure 6). One issue currently limiting the GNIP database is the spatial distribution of stations collecting precipitation information (as can be seen in Figure 6). The sample is far from homogeneous due to problems maintaining stations at high latitudes, altitudes and/or isolated stations such as those on small islands. 52% of stations are located within a 30^0 – 60^0 latitude band, and 72% are positioned at altitudes between 0 and 500m.

Figure 6. Geographical distribution of the meteorological stations belonging to the IAEA/WMO Global Network of Isotopes in Precipitation (GNIP).

Despite the limitations of the GNIP database, it has been established that the seasonal and spatial variation of the isotopic content of precipitation can be predicted over large geographical scales (regional, continental, global) (Aggarwal *et al.*, 2010; Bowen and Wilkinson, 2002). This allows scientists to anticipate the isotopic content of precipitation where no long-term data or observation stations exist. This in turn permits construction of continental-, country-, and even region-specific precipitation maps using GIS (geographic information systems) software.

The GNIP database and subsequent isotope precipitation maps are essential for use in forensic stable isotope profiling. Water is a vital component of dietary intake and is involved in many biosynthetic pathways; with its isotopic composition incorporated into body tissues (see Chapters 4 and 5). The isotopic content of these tissues can be analysed and compared with the ^{2}H and ^{18}O signature of precipitation. The isotope precipitation maps can then be utilised to suggest the geographical origin of an individual (Ehleringer *et al*., 2008). An increasing amount of research, particularly in the fields of ecology, hydrology, and forensic science relies on isotope precipitation information to establish the geographic origin of water or biological or geological materials. It is therefore

imperative that accurate global water isotope distribution maps are subject to regular updates and maintenance.

4.4 Isotopes in Plants

Plants are useful in forensic science both in terms of human identification and drug provenancing. The ^{13}C content of plant tissues can be analysed to determine whether it 'fixes' carbon dioxide via a C_3 or C_4 photosynthetic pathway, with each pathway providing plant tissues with a distinctive $\delta^{13}C$ value (Ambrose and Norr, 1993). This signature is incorporated into the animals and humans that consume them, either directly or indirectly (Cormie and Schwarcz, 1996). In essence this means that species consuming predominantly C_3 plants will display values reflecting the $\delta^{13}C$ ratio, as will any animals that feed upon them. In the same manner, body tissues of a C_4 consumer will reflect the $\delta^{13}C$ ratio of that group of plants. Some plants use a third carbon fixation method known as the CAM (crassulacean acid metabolism) pathway. Their tissues display $\delta^{13}C$ values between that of C_3 and C_4 plants, but contribute little to human diet. The ^{13}C content of plants with different photosynthetic pathways has been reported in many papers (Bender *et al.*, 1973; Szarek and Troughton, 1976), and is in general (these values are variable) as follows;

C_4 plants: around -9 to -18‰ (Bender *et al.*, 1973; O'Leary, 1988)
CAM plants: approximately -14 to -33‰ (Bender *et al.*, 1973)
C_3 plants: around -22 to -34‰ (Bender *et al.*, 1973; O'Leary, 1988)

Plants use the process of photosynthesis to 'fix' carbon, with the carbon source for all terrestrial plants being atmospheric CO_2 (Schoeninger, 1995). It is during this fixation process that the vast majority of isotope fractionation or discrimination occurs. The C_3 pathway is so called because plants in this group use an enzyme called ribulose bisphosphate (RuBP) to produce a phosphoglycerate compound with three carbon atoms as an intermediate product (O'Leary, 1988). The C_3 photosynthetic pathway is also known as the Calvin-Benson pathway, after the scientists who discovered it. Plants that can be found in the C_3 group include forest, montane and wetland grasses, all crops, vegetables, legumes, trees and shrubs, rice, wheat, and nuts and most fruits. In fact on a

global basis the vast majority of plants fall into this category. The C_4 (also known as Hatch-Slack) plants use an enzyme called phosphoenolpyruvate carboxylase (PEP carboxylase) produce a four carbon compound called dicarboxylic acid. C_4 plants include maize, millet, sorghum, sugar cane, some chenopods, setaria millets, some amaranths, and tropical pasture grasses. CAM plants can have $\delta^{13}C$ values that resemble either C_3 or C_4 plants, depending on the environment. This is because CAM plants PEP carboxylase to metabolise CO_2 when living in arid climates, but are also able to use RuBP. Cacti, agaves and euphorbias all use this pathway (Ambrose and Norr, 1993; O'Leary, 1988). In General, C_4 plants are adapted to hot, dry climates, with long hours of sunshine whilst C_3 plants often dominate shady areas that have a high winter rainfall, or are at high latitude or elevation. The CAM pathway is especially common in plants adapted to arid conditions such as succulents.

There are a number of factors that affect the level of carbon isotope fractionation in plants in addition to the photosynthetic pathway. These include light intensity, temperature, and water and nutrient availability. An example would be plants in regions of low rainfall having higher $\delta^{13}C$ values than those with an excess of water (Heaton, 1999). Another example would be the 'canopy effect' that, as a result of the combination of some of the factors mentioned, cause lower $\delta^{13}C$ values for animals feeding off the forest floor in comparison to those consuming food originating from higher in the canopy. This variation can range between 3 and 4‰ (van der Merwe and Medina, 1991). $\delta^{13}C$ values may also be affected by which part of the plant (e.g. leaves, seeds etc) is sampled. Variation of 1-2‰ have been recorded (O'Leary, 1981). Heaton (1999) has reported that seasonality also results in $\delta^{13}C$ variation, with differences of ±1‰ in plants depending upon the time of year they are sampled. Other factors include variation between species and forms, and regional changes. Variation in moisture, topography, and soil type can result in variation in $\delta^{13}C$ of up to 1.5‰. Altitude has also been shown to affect $\delta^{13}C$ values, increasing up to +1.5‰ per 1000m (Heaton, 1999).

The application of this information to the $\delta^{13}C$ value of unknown remains can be used to suggest their geographical origin (Meier-Augenstein, 2010). It has been established that the sole source of carbon used for the formation of human tissues is that of dietary intake

(Fogel and Tuross, 2003). Research investigating the $\delta^{13}C$ values of individuals originating from Europe, and those from North America discovered significant variation between the two. The majority of sugar consumed by individuals in Europe is derived from a C_3 plant called sugar beet, whereas North American sugar often originates from sugar cane and corn (C_4 plants) (Meier-Augenstein, 2010). Variation in the ^{13}C isotopic signatures of these two types of plant is incorporated into the body tissues of humans, and can be used to suggest geographical origin.

The information from stable isotopes of carbon is also of use to forensic scientists in drug-based investigations (Ehleringer *et al*., 1999). Research conducted by Ehleringer and colleagues (1999) investigating the provenance of heroin and cocaine indicated that samples taken from the four major growing areas for heroin (Mexico, South America, and South West and South East Asia) could be distinguished from one another based on a combination of carbon (influenced by humidity and rainfall) and nitrogen (influenced by soil type) isotopic signatures (see Figures 7 and 8). Ehleringer and colleagues (2000) have also suggested that isotopic profiling is precise enough that even slight variation in humidity levels can be used to differentiate correctly between cocaine that has been produced in Peru, Columbia, Ecuador or Bolivia (see Figure 9). Other studies support these findings, with the information provided by some investigators being utilised by law enforcement officers in Brazil to focus their efforts and reconstruct trafficking routes (Shibuya *et al*., 2006).

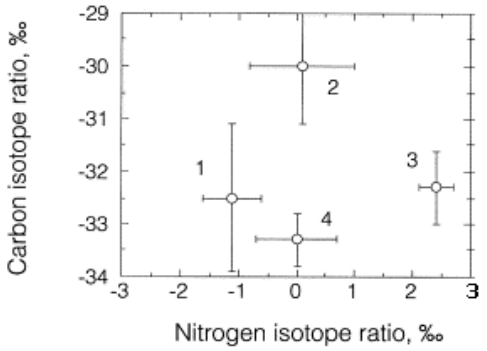

Figure 7. (Ehleringer *et al*., 1999). Carbon and nitrogen isotope ratios of authenticated heroin samples originating from the four major growing areas: Mexico (1), Southwest Asia (2), Southeast Asia (3) and South America (4)

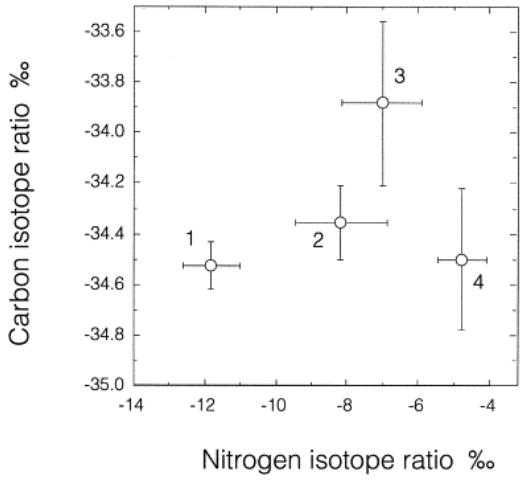

Figure 8. (Ehleringer *et al*., 1999) Carbon and nitrogen isotope ratios of authenticated cocaine samples originating from major growing regions in South America: Bolivia (1), Peru (2), Ecuador (3) and Colombia (4).

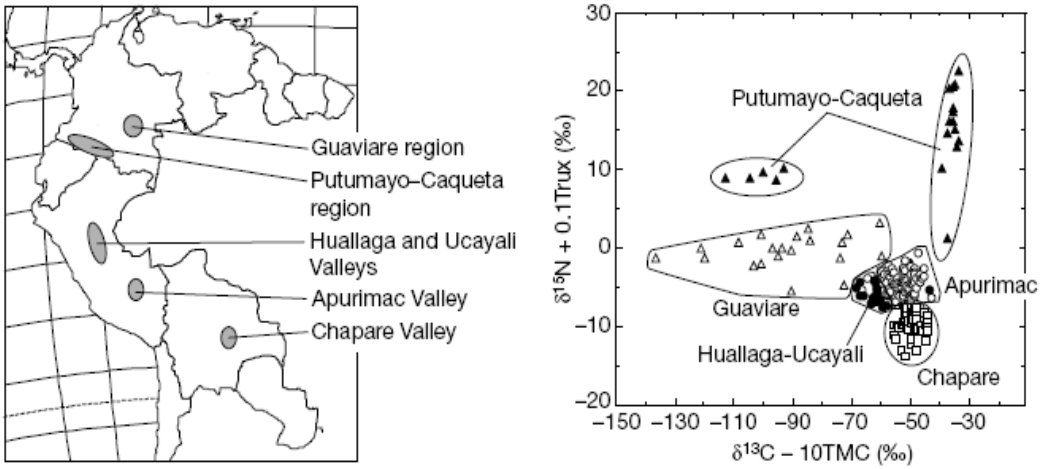

Figure 9. (Ehleringer *et al*., 2000) Geographical regions in South America producing illicit cocaine (left image); identification of cocaine-producing regions based on carbon- and nitrogen-isotope ratios and the abundance of minor alkaloid components truxilline (Trux) and trimethoyxcocaine (TMC) (right image); Bolivia (squares), Colombia (triangles), and Peru (circles). Regions within a country are shown by black and white symbols.

It is not only 'natural' drugs such as marijuana that can be used for stable isotope profiling, synthetic drugs such as ecstasy also exhibit isotopic ratio variations which

allow determination of the relationships among seized batches (Carter *et al*., 2002; Palhol *et al*., 2003). Carter *et al*. (2002) demonstrated, by plotting combinations of the hydrogen, carbon, and nitrogen isotope ratios of ecstasy tablets, that it is possible to identify groups or clusters that reflect different production routes for batches. This variation in isotopic signatures due to different production procedures allowed investigators to link specific seizures to specific manufacturers. Palhol *et al* (2003) demonstrated that it is possible to discriminate between seizure samples of ecstasy originating from a variety of geographic locations using their nitrogen isotope ratio values. In both of these examples the combination of stable isotope ratio values does not provide investigators with information regarding the geographical origin of the sample, but instead can assist in determining how many batches contributed to the seized samples.

Each stage of the drug production process introduces impurities into a batch, in the same way that laboratory analyses can be contaminated by impure chemicals, unsterilised equipment etc. It is also possible for acids and bases, and indeed water to become contaminated with ions and trace metals. The solvents used in production can carry organic contaminants, or may be contaminants themselves. Characteristic impurities within solvents can remain as a residue in the final salt product. The introduction of different solvents at a variety of processing stages adds to the impurities. As a result of the residual solvents being more likely to be found in higher concentrations than trace contaminants of reagents, it is these that are used in profiling methodologies (Cartier *et al*., 1997).

Chapter 5: Bone

5.1 Introduction

This chapter will provide a discussion of isotopic investigations using skeletal elements. There will be a brief account of research involving animals and the purpose of these studies, with the main focus of the chapter being the isotopic analysis of human bone. It will demonstrate the developmental and subsequent remodelling processes of human bone, and illustrate the incorporation of isotopic signals into the collagen and apatite fractions. A short section detailing the use of stable isotope profiling of skeletal elements in human identification will be included, discussing the data that currently exists and that which requires generation. The chapter concludes with an example of forensic investigations in which the stable isotope analysis of bone has assisted in resolving the cases.

5.2 Isotopic Research using Skeletal Elements

Much of the isotopic data collected from skeletal elements has been produced from the analysis of animal bones (Andrews and Nesbit-Evans, 1983; Longinelli, 1984; Luz and Kolodny, 1985; Luz and Kolodny, 1989; Schoeninger and DeNiro, 1984). The majority of isotopic studies involving bone have investigated food webs, and attempted to reconstruct both modern and ancient diet (Phillips and Eldridge, 2006; West *et al*., 2004). In human-based investigations a substantial amount of data has been collected from ancient remains and used to reconstruct dietary intake, establish seasonal migratory patterns, determine the introduction of maize to various civilisations, and infer the social status of an individual within a community (Macko *et al.*, 1999; Tieszen and Fagre, 1993; Van Der Merwe *et al.*, 2003; Van Der Merwe and Vogel, 1978).

In isotopic terms, ‘you are what you eat’ in that the isotopic values of an individual’s dietary intake are incorporated into their body tissues. This can be either directly (by consumption, for example, of a primary producer) or indirectly (by consumption of, for example, a herbivore that has fed on a primary producer) (Hedges and Reynard, 2007). When subject to body processes such as digestion, the isotopic signatures of the food (or water) alter slightly due to fractionation (Ambrose and Norr, 1993; Chisholm, 1989).

This variation passes through the food chain from primary producer to consumer (which may be several trophic levels; primary producer to herbivore, to carnivore, to human), with fractionation processes occurring constantly and differing according to factors such as metabolism (Ambrose and Norr, 1993).

It has been suggested that in humans, the variation between $\delta^{13}C$ values of diet and collagen is +5±1‰ (Keegan and DeNiro, 1988). Other literature seems to confirm this as a general average, for example Ambrose and Norr (1993) found that humans on natural diets and large free ranging animals have fractionation values of between +4.7 and +6.6‰; van der Merwe and Vogel (1978) quote a fractionation value of +5.1‰ for human bone collagen. The issue with using these values in forensic investigations is that they were calculated using data collected from ancient skeletal elements, and may not be relevant for modern day human remains. Factors such as variation in metabolism (and hence activity levels) may affect fractionation levels and could therefore cause these values to differ.

The use of isotopic information originating from ancient populations in modern forensic investigations may result in confusing or incorrect conclusions. One explanation could be the 'globalisation' of dietary intake. Many foods are now imported from a variety of countries, and society is exposed to a number of products that were either not available to ancient civilisations (depending upon geographical location), or were only accessible during certain seasons. The consumption of crops grown in a variety of locations (that will display the isotopic signature of the region in which they were produced) could result in a puzzling analytical output. The freedom of modern populations to travel great distances may also lead to the generation of confusing stable isotope profiles. It is likely that individuals will be consuming the local water and produce of the area in which they are staying. These isotopic signatures will become incorporated into their body tissues, and may 'blur' other signatures indicating their primary geographical origin. Ancient populations also lived in small communities, with each individual consuming a very similar diet to all others in a community, and migrating with these individuals. It is likely that any attempts to use data from these studies for quantifying inter-individual variability will demonstrate smaller error values than with modern populations. The variety of foods

(and hence isotopic signatures) available to modern humans is substantially greater than ancient populations, and therefore the isotopic composition of modern human tissue is likely to display a greater variability. This evidence illustrates the requirement to collect isotopic data from contemporary individuals in order to assess and quantify the levels of inter- and intra-individual variability for forensic purposes effectively.

Another issue with the use of ancient material (either human or animal) for analysis is that it may have undergone diagenetic alteration, and provide erroneous data. Authors have expressed concern over the possible diagenetic effects on bone (Collins *et al*., 2002), but these factors are currently relatively unexplored. However, it has been established that the composition and quantity of surviving organic material in skeletal elements are dependent upon their burial environment (Collins *et al*., 2002). Suggested environmental factors influencing the degradation of collagen include pH, temperature, and alteration brought about by soil flora and fauna (Tuross *et al*., 1988; van Klinken, 1999). In cool, stable conditions collagen can be well preserved, Ambrose and Norr (1993) report that the isotopic composition of collagen can remain intact up to 80-100,000 years after burial. It has been found however that hot, dry, and exposed burial sites are not conducive to preservation. Factors known to alter the isotopic signature of the apatite fraction include dissolution of the mineral by acidic rainwater, temperature extremes, and microbial activity (Collins *et al*., 2002; Lee-Thorp and Sponheimer, 2003). The diagenesis of bone apatite involves a process called recrystallisation. This is where the usually small and poorly organised apatite crystals begin to fuse to become larger and more organised (Schoeninger, 1982; Tuross *et al.*, 1989). During the recrystallisation process carbonate and phosphate ions in solution may be incorporated into the apatite lattice. Stable isotope analysis of diagenetically altered bone apatite may include these ions which are unrelated to biogenic values, and therefore resultant data may be misleading. The diagenesis of both collagen and apatite is not solely exclusive to ancient remains, but is also likely to affect modern material that has been buried or exposed to extreme environmental conditions. It is therefore important for this potential error to be considered, and accounted for, particularly when collecting evidence for forensic investigations.

Isotopic data on bone originating from modern populations is particularly scarce, as the collection of samples is highly invasive and is only performed on deceased individuals. The majority of isotopic studies utilising more contemporary human samples are from forensic cases where there may be little or no background information on the individual against which to compare the results. Another issue associated with the application of this type of data is that there may only be certain skeletal elements available for analysis as a result of, for example, scavenging by carnivores. It is well documented that bones have varying turnover rates (Hill, 1998), making it inappropriate to compare isotopic signatures directly from different elements. The literature documenting inter- and intra-individual variability is also limited, meaning the interpretation and comparison of data collected from remains of differing age, sex, and ancestry can be problematic. It is essential that these potential errors are explored further and are quantified.

This research intends to address some of these issues by establishing both the inter- and intra-individual variability associated with modern isotopic bone carbonate data, while considering factors such as age, sex, and remodelling rate. It will also provide error values essential for the admissibility of isotopic evidence in a courtroom environment.

5.3 The Human Skeleton

The human skeleton is comprised of single and fused bony elements, held together by ligaments, tendons, muscles, and cartilage. It acts to support and protect vital organs, such as the heart and brain. It also serves as an anchor for muscles and a store for minerals, in particular calcium, which is essential for functions such as electrical conduction of the heart, and neurotransmitter release. There are two main types of bone, trabecular and cortical which differ both in appearance (see Figures 10 and 11) and role. Trabecular bone (also called cancellous or spongy bone) typically occurs at the end of long bones (see Figure 12) and in flat bones like the pelvis. It is more flexible than cortical material, and is responsible for distributing and dissipating the energy from mechanical loading on the bone (Pate, 1994). Cortical bone (also called compact bone) is more dense than trabecular bone, and is found primarily in the shaft of long bones (see Figure 12). It forms the outer shell surrounding trabecular bone at the ends of joints and

vertebrae. It is highly organised into cylindrical elements called osteons which are composed of concentric lamellae (see Figure 16). It is comprised of a cellular component composed of osteoblasts (bone-forming cells), osteoclasts (bone-removal cells), and osteocytes (bone maintaining-cells) which are inactive osteoblasts incorporated into the extracellular matrix (Pate, 1994). This highly organised structure assists cortical bone in providing the mechanical strength of the skeleton.

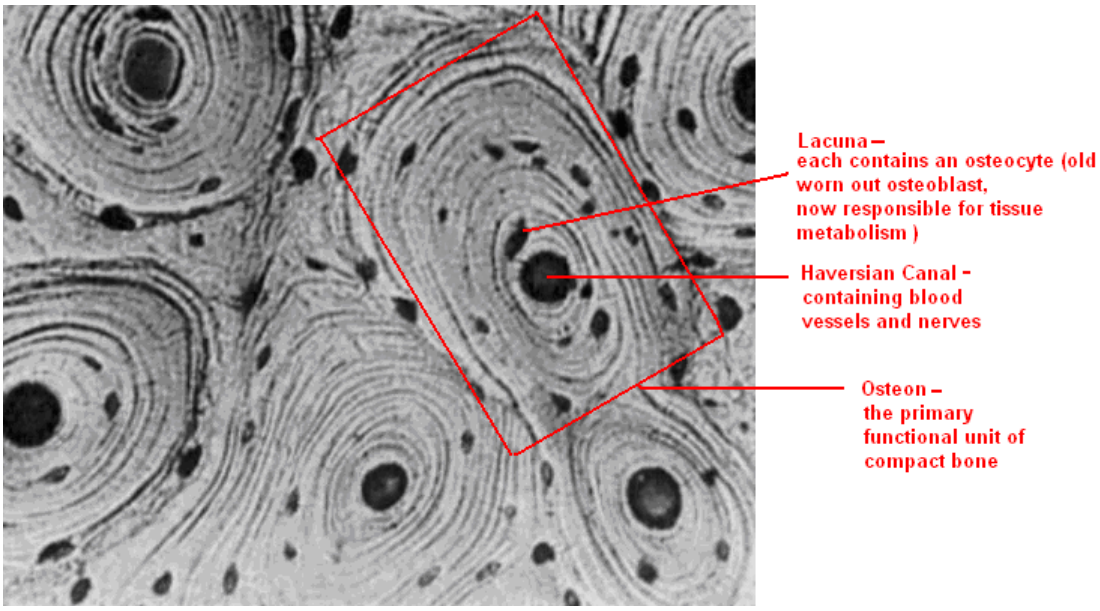

Figure 10 . (ICB-DENT, 2010). An image demonstrating the microscopic structure of cortical bone

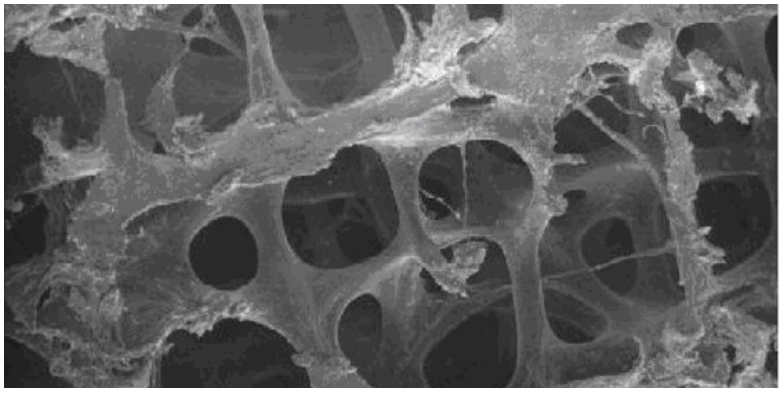

Figure 11. (ICB-DENT, 2010). Microscopic image illustrating the trabecular structures inside a first lumbar vertebra.

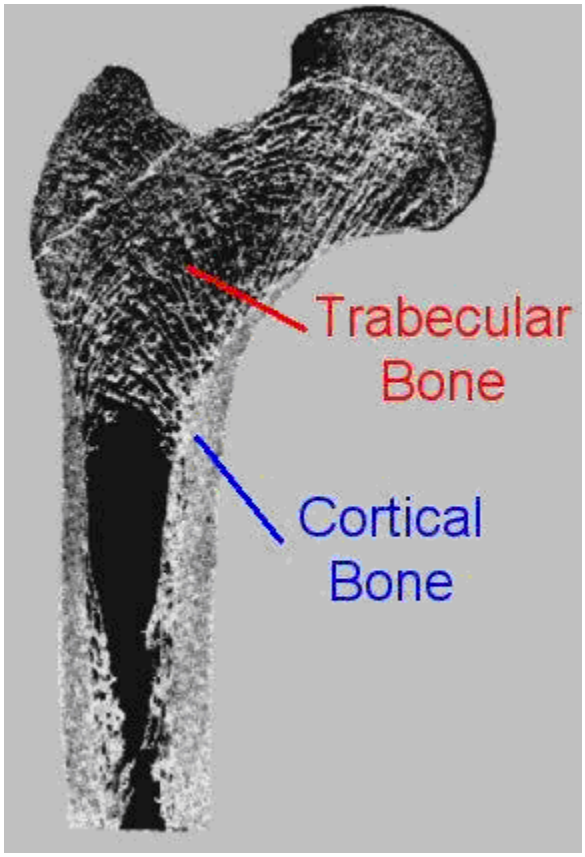

Figure 12. (ICB-DENT, 2010)**.** An image of a longitudinal section of a human femur.

This research utilises the cortical element of a bone, and therefore shall focus its discussion on this area, as opposed to trabecular material. Bone is a connective tissue comprised largely of an organic protein called collagen, and an inorganic mineral, hydroxyapatite.

Compact bone is comprised of inorganic calcium phosphates inside an organic collagen matrix. It is approximately 69% inorganic, 22% organic and 9% water (Pate, 1994). Around 90% of the organic portion of the cortical bone is comprised of the protein collagen. The organic fraction is comprised mainly of collagen fibres, and the inorganic mineral portion of hydroxyapatite crystals (Holden *et al*., 1995). Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), which includes calcium carbonate, calcium phosphate, calcium hydroxide, calcium fluoride, and citrate is predominantly crystalline in structure, although it may also be present in amorphous forms (Hedges and Van Klinken, 1992). The hydroxyapatite portion acts as a mineral reservoir and frequently exchanges mineral

ions with body fluid (blood). Supplementing ions from nutritional intake, are also introduced to the structure, either replacing minerals that are depleted or being adsorbed on to the crystal surfaces (Rolla and Bowen, 1978). Since dietary water is the major source of oxygen for hydroxyapatite (Meier-Augenstein, 2010), the addition of ions from nutritional intake to the hydroxyapatite, results in the incorporation of dietary isotopic signatures into bony tissues.

Research has suggested that bone collagen and bone carbonate reflect different dietary components (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). This results from the fact that collagen is composed of both essential and non-essential amino acids. Essential amino acids arise solely from ingested protein, and non-essential amino acids are formed either from ingested protein or from other dietary sources (Burton, 2008). Bone carbonate is formed from blood bicarbonate (bicarbonate dissolved in the blood), which comes from ingested carbohydrates, proteins, and lipids. Therefore, the carbon within bone apatite is indicative of the total diet, whereas collagen reflects ingested protein (Ambrose and Norr, 1993; Burton, 2008; Tieszen and Fagre, 1993). Both collagen and apatite constantly undergo renewal (see later section), meaning that their isotopic signatures reflect an individual's dietary intake over the previous years. The amount of time represented is dependent upon the skeletal element under investigation (as remodelling rate varies), and can range from 5-10 years for human ribs (Hill, 1998), up to around 25 years for human femora (Carter, 1984).

Bone remodelling is the removal of old inactive bone from the skeleton, and its replacement with new tissue. Adaptation and remodelling occurs in response to stresses and strains on the bone resulting from factors such as loading, trauma, and disease. Wolff's law (a theory developed by Julius Wolff, a German anatomist/surgeon) states that bones in healthy individuals will adapt to the loads they are subjected to (Chamay, 1972). Although Wolff's law has been challenged (Bertram and Swartz, 1991; Pearson and Lieberman, 2004), its basic principles are still upheld (Ruff *et al.*, 2006). If the load placed on a particular skeletal element increases, the bone develops a structure designed to resist and distribute the stress. Both the internal architecture of the trabeculae and the external cortical portion undergo adaptive alterations in response to the variation in

external loading conditions (Chamay, 1972). The changes follow, precise mathematical laws.

When variation in loading pattern occurs the bone tissue alters accordingly. The internal architecture changes in terms of density and disposition of the trabeculae and osteons. The cortical element adapts in terms of shape and dimensions. As the strain on bone intensifies, new tissue is formed. The process of removal and addition of bone tissue is called remodelling, and is performed by the cellular components of bone tissue (Hill, 1998). Resorption involves the breakdown of the collagen and mineral phase (see Figure 13) by osteoclasts. The products of this are then removed by the circulatory system and either utilised or disposed of by the body. During deposition of new bone osteoblasts converge on the surface where the new tissue will be formed, and build a collagen network of bone (see Figure 14). Mineralisation of the collagen matrix occurs afterwards.

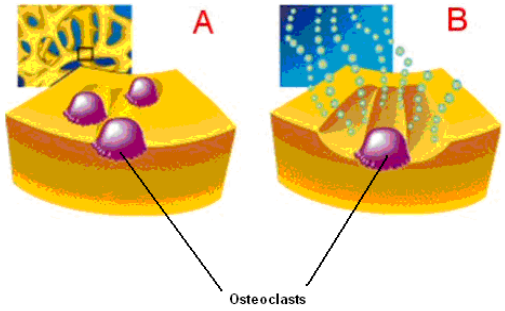

Figure 13. (ICB-DENT, 2010). The process of resorption, performed by osteoclasts. Osteoclasts resorb both the collagen and mineral portions (A) which are then taken up by the circulatory system (B).

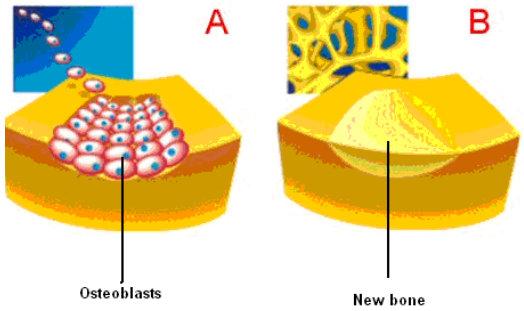

Figure 14. (ICB-DENT, 2010). The process of deposition, performed by osteoblasts. During the deposition process osteoblasts cluster on the deposition surface and lay down a new collagen network (A). Mineralisation of the occurs later (B).

Bone resorption and deposition constantly occur in skeletal elements. Equilibrium exists where the two processes are perfectly balanced, unless factors such as disease or trauma are introduced (Guise and Mundy, 1998; Hill, 1998). Remodelling is a dynamic and constant event that is rarely in equilibrium; when increased strain is placed on the bone the equilibrium shifts, deposition activity decreases, and net resorption occurs (see Figure 15). Equilibrium returns once the bone has strengthened enough to withstand the increased strain imposed. The activity of osteoblasts and osteoclasts in formation and resorption of bone is regulated by factors such as genetics, hormones, and metabolic processes (Brixen *et al*., 1990; Guise and Mundy, 1998).

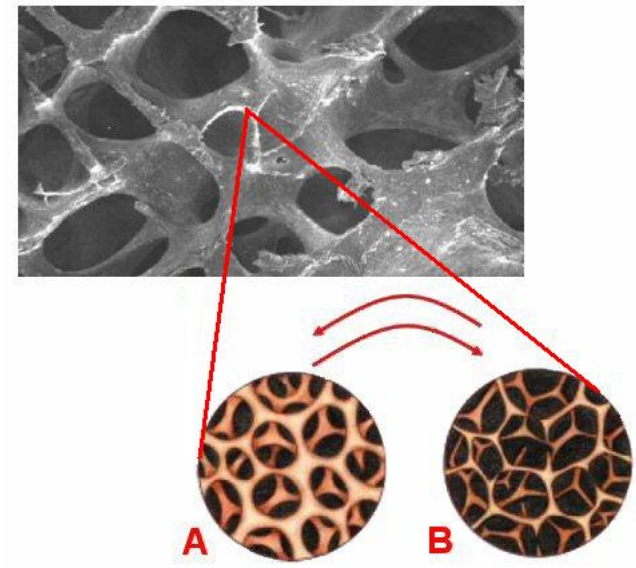

Figure 15 . (ICB-DENT, 2010). The effect of reduction (A to B) and intensification of strain (B to A) on bone trabeculae.

The dependence of remodelling on factors such as injury, disease, and metabolism means that it is highly variable and may result in different isotopic values of bone tissue. Estimates for the turnover rate of collagen vary considerably from 2 to 30 years (Chisholm, 1989). The turnover rates of cortical bone are also highly variable and as mentioned, dependent upon the skeletal element under investigation. Recent investigations by Hedges *et al.* (2007), and Ubelaker *et al.* (2006) suggest that there is a substantial amount of variation in annual collagen turnover rates depending upon the sex

and age of the individual under observation. This can range from as much as 30% per year in adolescent males (younger than 19 years old) to as little as 1.5% per year for males in their 80s (Ubelaker *et al*., 2006). Research investigating the turnover rate of cortical bone also indicates that the rate is sex and age dependent (Carter, 1984).

Ubelaker *et al.* (2006) studied the collagen from two females in their 70s and discovered that on average the ^{14}C isotopic composition reflected their diet some 40 years previous, although there had been new collagen synthesised. Hedges *et al.* (2007) discovered that at 50 years old an individual's collagen can contain up to 40% that was synthesised prior to 25 years of age. They draw the conclusion that the isotopic signature of human femoral collagen reflects an individual's dietary intake over a longer period of time than 10 years, and includes a significant amount of collagen synthesised during adolescence (Hedges *et al*., 2007). Other studies have also suggested that the carbon and nitrogen isotope signals from both collagen and apatite will reflect an average of the diet (Jim *et al.*, 2004; Sealy *et al.*, 1995).

As previously stated, this is dependent upon the skeletal element under investigation, and can range from 5-10 years for the rib (Hill, 1998), to approximately 25 years for the femur (Carter, 1984). This long residence of isotopic signatures in the bone tissue of adults means any dietary variation will not be immediately apparent, and the isotopic composition can be viewed as a dietary average of what the individual has consumed over a number of years. Some studies have suggested that not all skeletal elements remodel constantly throughout life; for example the petrous portion of the temporal bone does not undergo further remodelling after the age of two years (Frisch *et al.*, 2000). Jørkov and colleagues (2009) found that the carbon and nitrogen isotope composition of the petrous portion of the temporal bone of 34 adults and 24 subadults reflected that of the 1st molar which is formed early in life, and the isotopic composition of which reflects dietary intake during childhood and early adulthood. The carbon and nitrogen isotope composition of the petrous portion of the temporal bone were also found to be significantly different from that of the rib (turnover rate of 5-10 years) and femur (turnover rate of ~25 years) from the same individual (Jørkov *et al.*, 2009). These results

demonstrate the importance of sampling a variety of skeletal elements for stable isotope profiling, and the possibility of using the petrous part of the temporal bone for estimating the dietary intake of an individual during childhood and early adulthood.

These are important concepts when investigating the stable isotopic composition (H, C, O, N) of human bone tissue in forensic casework. There is very little literature available detailing remodelling rates, and the studies that have been performed either focus on ancient material, or are medical-based studies using subjects presenting with disease or trauma. It is essential that errors associated with turnover rates are acknowledged and quantified, so that the stable isotope analysis of human bone tissue becomes a robust and reliable forensic technique. This research intends to provide data that considers the differences in bone tissue turnover rates by quantifying the intra- and inter-individual variability associated with isotopic analysis.

5.4 Stable Isotope Analysis of the Human Skeleton as a Forensic Technique

The use of oxygen isotope analysis on skeletal material to reconstruct migration patterns, ancient climates, and the origins of both ancient humans and animals has been extensively researched (Iacumin *et al.*, 1996; Kohn *et al*., 1996; Wright and Schwarcz, 1998); it is the use of this technique for forensic investigation that is novel. Application of isotopic analysis to modern human skeletal material is a recent development with regard to forensic science. Peer-reviewed, published literature detailing the forensic applications and utilisation of isotopic signatures in modern skeletal remains is scarce. In particular, there have been very few studies conducted utilising the isotopes of oxygen for inference of geographical origin and regions of residence (Pye and Croft, 2004).

Despite a lack of scientific research, it has been established that isotopic profiles extracted from human skeletal elements can be used to reconstruct the geographical history of an individual, with the method applied in several forensic cases (Meier-Augenstein, 2010). The majority of oxygen atoms in our bodies originate from the water we consume, which tends to be isotopically similar to the precipitation in the area an

individual resides (Ehleringer *et al.*, 2008; Fraser and Meier-Augenstein, 2007; Fraser *et al.*, 2006). From careful examination of bone samples and the use of equations developed by geochemists (see Chapter 7) to determine the likely $\delta^{18}O$ value of the drinking water consumed (Daux *et al.*, 2008; Longinelli, 1984), the skeletal elements of an individual can be used to determine their geographical origin. In a similar fashion, the only source of carbon for construction of human tissue is that from dietary intake (Fogel and Tuross, 2003). Investigation of the carbon isotopic signature of individuals originating from Europe, and those from North America reveal significant differences (Meier-Augenstein, 2007). This is a result of nutritional sugar-based variation. The majority of sugar in North American diets originates from sugar cane and corn, with the latter also used to feed livestock (Meier-Augenstein, 2010). Many processed foods also contain corn syrup, which can be found in beverages such as beer and wine (Brooks *et al.*, 2002; Wagenmakers *et al.*, 1993). In contrast the majority of sugar within the dietary intake of Europeans originates from sugar beet, a C_3 plant (Wagenmakers *et al.*, 1993). Sugar cane and corn are C_4 plants, and have very different isotopic signatures when compared with C_3 types such as sugar beet (see Chapter 4 for reasoning) (O'Leary, 1981).

Bone is constantly remodelling throughout a person's lifetime. As a result isotopes record the location(s) an individual has resided in for the past 10-20 years. As an individual ages, the rate and extent to which remodelling occurs tends to decline, although injury or stress to an element will increase the remodelling rate (Carter, 1984; Chamay, 1972). Tooth enamel once formed (unlike bone) does not undergo remodelling (Wright and Schwarcz, 1998). Since the majority of tooth enamel is constructed either before birth or during adolescence, the $^{18}O/^{16}O$ and $^{2}H/^{1}H$ content of teeth records the geographical location of an individual at the time of tissue formation. Of particular interest are the second and third molars, as they are late erupting and will record geographical location during adolescence. Other teeth such as premolars are formed early in life and will retain signatures resulting from the weaning process (i.e. incorporation of the mother's isotopic signature) (Wright and Schwarcz, 1998).

Using maps generated by $^{18}O/^{16}O$ and $^{2}H/^{1}H$ precipitation information and GIS software, it is possible to consider the region in which an individual might have resided. These

maps are widely available and illustrate the global distribution of $^{18}O/^{16}O$ and $^{2}H/^{1}H$ (Bowen and Wilkinson, 2002), and more specifically isotopic distribution in the USA and UK (see Figures 16 and 17) (Darling *et al*., 2003; Darling and Talbot, 2003; Ehleringer *et al*., 2008). In general ^{18}O content increases from the poles to the equator, and from the interior of a continent to the west coast (due to the movement of weather patterns from east to west). Precipitation from mountainous regions also displays depleted ^{18}O content (Gourcy *et al.*, 2005). The information collected through stable isotope analysis can be compared to these maps, and can assist scientists in tracing the possible origin of an individual such as a murder victim (Meier-Augenstein and Fraser, 2008).

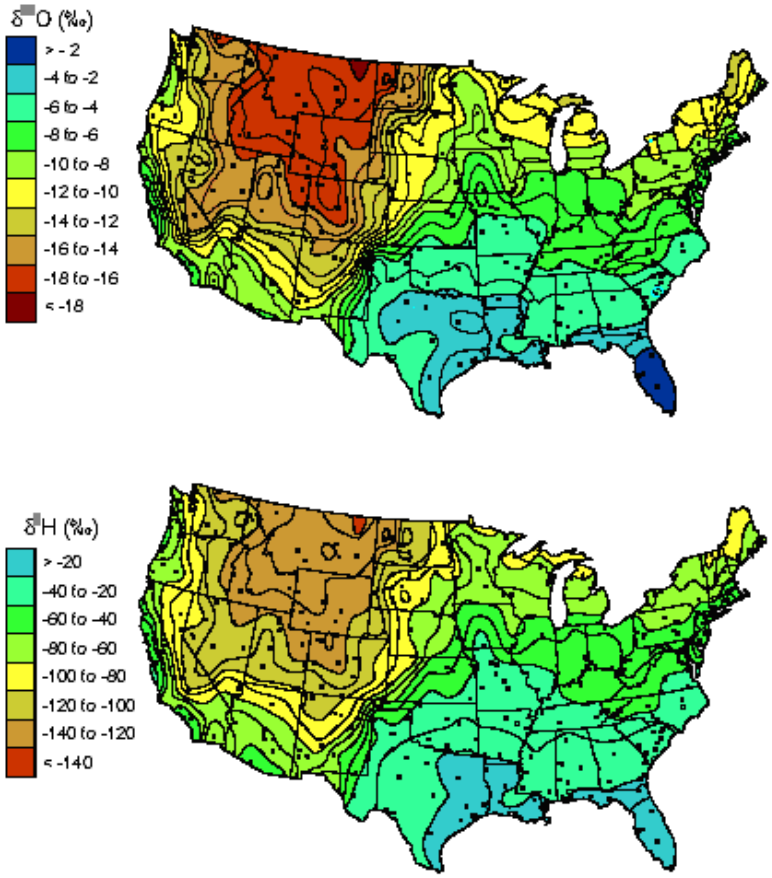

Figure 16. (Schwarcz, 2007). Oxygen and hydrogen isotope map of North America. Brown to yellow colours denote regions in which $^{18}O/^{16}O$ and $^{2}H/^{1}H$ is low, and green to blue areas where $^{18}O/^{16}O$ and $^{2}H/^{1}H$ is higher.

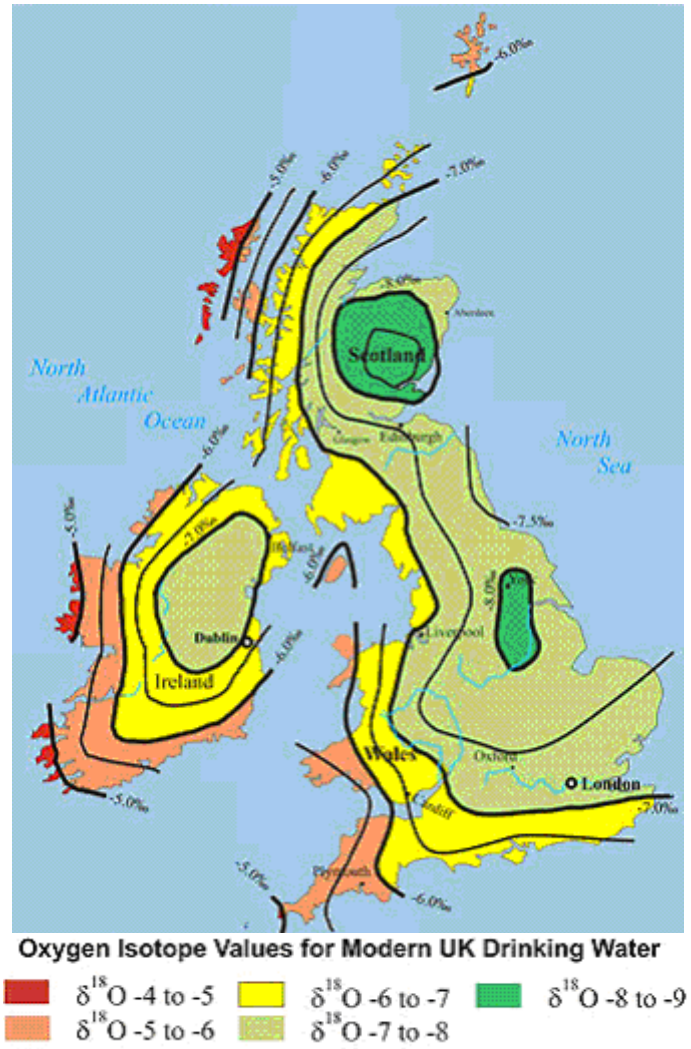

Figure 17. (NERC, 2010). Oxygen isotope map of the UK. Green to brown colours denotes regions in which $^{18}O/^{16}O$ is low, and pink to red areas where $^{18}O/^{16}O$ is higher.

Although the maps demonstrate a substantial number of locations an individual could acquire a particular ^{18}O or ^{2}H value, the isotopic content of body tissues are still a powerful identification tool, particularly when combined with other information. For example, if remains are discovered at a particular location, isotopic analysis could be applied to establish whether they resided in that particular region or whether they were just visiting. If the isotopic values of body tissues can be clearly differentiated from local values, it is reasonable to conclude that the individual is not from the area, and other possible locations of origin can be examined. The individual may have resided in a

number of geographical regions throughout their lifetime (infancy, adolescence, adulthood). It is therefore vital to sample a number of body tissues (if available), with a variety of formation rates, to determine a full geographical history.

5.5 Research Purpose and Rationale

As discussed in the previous section, there is a requirement for further study of stable isotope profiling for use in human identification. There is particular need to investigate the variability both between and within individuals that may result from factors such as metabolism and dietary preference. The research presented in this thesis intends to address this by quantifying the inter- and intra-individual variability associated with bone derived data. This shall be achieved by studying the ^{13}C and ^{18}O content of bone apatite, and using the data in a number of ways;

1. To quantify the variation in $\delta^{13}C$ and $\delta^{18}O$ values between several individuals
2. To assess the level of variation in isotope content between the left and right legs from a single individual
3. To quantify the variation in the ^{13}C and ^{18}O content of a single sample analysed on different days
4. To establish the variation in $\delta^{13}C$ and $\delta^{18}O$ values between a number of samples collected from the same femoral section
5. To assess whether the variation in the isotope content of a sample is attributable to the location (on the femoral section) from which it was taken.

To achieve these aims transverse femoral sections will be harvested from both right and left legs of cadavers donated to The University of Dundee for educational purposes. Femoral sections submitted to the laboratory for forensic investigation will also be analysed. Several holes will be drilled within each section, and the resultant powdered bone subject to stable isotope analysis. The results of stable isotope analysis will be used to quantify the levels of inter- (comparing the data collected from different individuals) and intra- individual (comparing the data collected from each of the holes drilled in one bone section from one individual) variability. It is also envisaged that dietary preferences

(relating to a predominantly C_3 or C_4 plant diet) may be deciphered through the resultant data. In addition, the information will be used to estimate the geographical provenance of those samples of unknown origin (forensic casework). For those samples of known origin (the University of Dundee cadavers) the $\delta^{18}O$ values collected from bone carbonate will be converted to the likely delta value of drinking water consumed. The estimated delta value of drinking water will be used to confirm geographical provenance by comparison with isotope maps for tap water produced by Darling and colleagues (2003). Research has already demonstrated two important relationships that are acknowledged by this study:

1. There is a strong link between oxygen isotope ratios in skeletal tissues and the oxygen isotope signatures of ingested water (Iacumin *et al.*, 1996; Levinson *et al.*, 1987).
2. There is no significant difference between the isotopic content of local tap water and the isotopic signature of local precipitation (Bowen *et al.*, 2007).

Although the link between the isotopic content of tap water and precipitation is based on research performed in the US, there are oxygen and hydrogen stable isotope composition maps available for precipitation in the British Isles (Darling *et al.*, 2003; Darling and Talbot, 2003). The analysis of carbon isotopes in bone carbonate are important in determining the dietary intake of an individual, as literature has demonstrated that carbon isotopes can be used to determine whether a diet consists of predominantly C_3 or C_4 plants (see Chapter 4 for description of plant types) (Meier-Augenstein, 2010). It is therefore reasonable to expect to extract information regarding an individual's geographical location and dietary intake through the analysis of stable carbon, and oxygen isotopes from their tissues, as shall be performed in this research.

Chapter 6: Method and Materials

6.1 Collection and Preparation of Human Bone Samples

Bone samples utilised in this research were all femoral sections either collected by the researcher, or sent to the Scottish Crop and Research Institute (SCRI) stable isotope laboratory for analysis as part of a forensic investigation. It is standard procedure for the laboratory to request that samples collected for isotopic analysis be mid-shaft femoral sections of around 1-2cm in length. Femoral sections are preferred as they record dietary intake over a longer period of time than other skeletal elements (Carter, 1984). It is also a large weight bearing bone, and provides investigators with a sufficient amount of sample for multi-elemental analysis in triplicate.

A total of 4 femoral sections were collected from 3 cadavers used for human dissection at the University of Dundee. The individuals sampled had given prior consent and the sections of bone were removed and stored in accordance with the Human Tissue (Scotland) Act of 2006. The majority of people choosing to donate their bodies for research are elderly, and therefore a large proportion of the material is from individuals over the age of 70 years. The sex, cause of death, and most recent region of residence (this could be a hospital or care home) is documented for each individual (see Appendix 1). The cause of death may not always be absolutely definitive and there may be conditions present that would affect bone (for example a tumour) that may not be recorded in official documentation. In order to gain access to the femora, soft tissue was removed from around the mid-shaft of the bone using a scalpel. Transverse cuts were made in the femora using a using a Stryker® autopsy saw (designed specifically to cut through bone and plaster casts), and a section of around 1-2cm in length removed. Where possible sections were collected from both the left and right femora, however if an individual had a hip replacement, the prosthesis extends into the mid-shaft region and sections could not be removed. In addition, if there was obvious disease or traumas affecting the bone, femoral sections were not taken. This is due to the fact that injury and stress to bone tissue results in increased remodelling (Chamay, 1972). The newly generated tissue in the region of injury or stress will have an isotopic content reflecting dietary intake during formation, and may mask the signature of the original, older bone.

Once removed from the cadavers, sections were placed in plastic bags labelled with the cadaver number, and L or R to indicate whether the sample originated from the left or right femur. Sections were then prepared for the drying process. Preparation involved using a combination of tweezers and haemostats to remove any remaining soft tissue, and scrape out the contents of the marrow cavity. Sodium hypochlorite (NaOCl) is often applied to bone material as a degreasing agent but was not used in this study; this was because NaOCl may have introduced chemical contamination to the sample. It was important to remove all soft tissue to speed up the drying process and rid the sample of potential organic contaminants (as this research is focused on the inorganic component). Bone sections were then removed from their plastic bags and labelled using cadaver tags (these were numbered and marked with either an L or R). This was achieved by feeding a length of string through the marrow cavity and attaching the tag to the string. The sections were selected and placed in an evacuated desiccator over self-indicating phosphorous pentoxide (Sicapent®), a powerful drying agent. The 'spent' layer of Sicapent® (i.e. that which has absorbed the moisture) was removed every other day to allow further absorption of excess water. It is essential that prior to analysis all samples have any associated moisture removed. This is because isotopic analysis will provide data from the ^{18}O content of water within the sample, rather than the bone phosphate. In an attempt to monitor the drying process bone sections were weighed once every two days and their weight loss recorded. Once the loss in weight had reached a plateau, it was reasonable to conclude that any excess moisture had been removed, and sections were ready for further sampling.

Three bone sections of unknown provenance were sent to the laboratory as part of forensic casework, and were subsequently analysed for their isotopic signature. One bone section with no associated history was sourced from the University of Dundee teaching collection, and also analysed for its isotopic content.

6.2 Collection of Dry Bone Material for Analysis

Once all moisture had been removed from a femoral section, small subsamples were taken in the form of powdered bone. Multiple holes were drilled in the bone section (see

Figure 18), using a Dremel® Multi Drill with a 1mm diameter tip which was cleaned between each sample using methanol to avoid cross-contamination. The amount of sampling sites on each bone section was dependent upon the width of the cortical bone from lateral to medial. Some sections permitted the drilling of two or three holes from lateral to medial across the bone (for example X65 D10, Figure 24), other sections were too thin and only had one site sampled (for example 792L, Figure 29). During the drilling process it was important to avoid both the inner and outer cortex of the bone. The reasoning behind this is that the outer cortex previously had muscle tissue attached and the inner cortex was enclosing the contents of the marrow cavity, both of which are organic materials. This research is focused on the inorganic component of bone (bioapatite), and any organic contaminants within the sample could distort the subsequent isotopic signature. It was also essential the Dremel® drill was kept at the lowest speed possible so as not get hot and potentially cause isotope fractionation with the small samples being collected. The powdered bone extracted from each hole was collected in tin foil 'boat' (labelled with bone sample details and drilled hole number) and stored in a drying oven until commencement of the next preparatory stage. Multiple samples were taken from the same femoral section to allow for subsequent statistical analysis of intra-individual variability. For triplicate analysis (3 repeat analyses of the same hole) at least 12mg (4mg per repeat) of bone bioapatite must be drilled from the bone, although 6-7mg is preferred in order to produce clearer, well defined peaks on the analytical output.

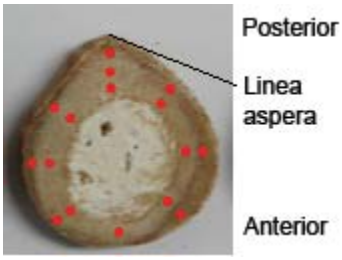

Figure 18. Example of a femoral section with the location of drilled holes indicated by red dots.

6.3 Preparation of Carbonate from Bio-apatite for Isotopic Analysis of ^{13}C and ^{18}O

Approximately 5-7mg of powdered bone was weighed, using a Sartorius Research microbalance, into an Exetainer® (Labco, High Wycombe, United Kingdom). This was carried out by placing Exetainer® on the balance, and then taring it. Sample was removed from the tin foil 'boat' using a sterilised (with methanol) spatula which was cleaned using methanol between each sample, and transferred to the Exetainer®. Two sample repeats were weighed out for the same hole and the Exetainer® labelled with the bone section details and either 'A' or 'B' (denoting the two repeats). Six Exetainers® were filled (using the same procedure) with 0.5mg of standard, these included (2x) the international reference material NBS-19 ($\delta^{13}C_{VPDB}$ = +1.95‰; $\delta^{18}O_{VPDB}$ = -2.20‰), (2x) the international reference material (x2) LSVEC ($\delta^{13}C_{VPDB}$ = -46.6‰; $\delta^{18}O_{VPDB}$ = -26.7‰), and (2x) an in-house standard called Bicarb-X ($\delta^{13}C_{VPDB}$ = -4.5‰; $\delta^{18}O_{VPDB}$ = -12.17‰). These standards were chosen as NBS-19 and LSVEC act as 'anchors' at either end of the $\delta^{13}C$ and $\delta^{18}O$ scales, with the reported value of Bicarb-X falling between the two. This anchoring allows comparison of isotope data from laboratories around the globe. Standards are also important as their known values can be used to establish quality of the other results. Two other empty Exetainers® were used in the analysis and were labelled as blank (contains no sample) and acid blank (contains no sample, will eventually contain sulphuric acid). These are also used as quality control indicators.

It is essential that all traces of atmospheric CO_2 are removed from the Exetainers®, as its isotopic content will be analysed in addition to that of the gases from bone carbonate. This removal of ambient CO_2 was performed by introducing N5.7 grade nitrogen (N_2-BIP; Air Products, Crewe United Kingdom) at high pressure to each Exetainer® (all samples, references, and both blanks) through its septum, for eight minutes. Blank Exetainers® were flushed and subsequently analysed for CO_2 content, before any Exetainers® containing sample or reference material. This was undertaken to confirm the technique of removing atmospheric CO_2 using high pressure N_2 was producing the desired result.

Once all Exetainers® had been flushed, an acid digest was performed to evolve the CO_2 content of the bone carbonate (see equation 1). 0.5ml of water free (absolute) sulphuric acid (99.999%) was added to each Exetainer® (all samples, references, and the acid blank) by injection through the septum using a Greatcare Med sterile disposable syringe combined with a BD Microlance™ sterile needle (0.8mm x 40mm), with a new syringe used for each Exetainer®. Water free sulphuric acid was used as opposed to absolute phosphoric acid as it was readily available in the SCRI laboratories.

$$CaCO_3 + H_2SO_4 \rightarrow CaSO_4 + CO_2 + H_2O \qquad (1)$$

In an attempt to stop the addition of atmospheric CO_2 during this process, 0.6ml of acid was drawn up into each syringe but the plunger only depressed until 0.5ml was gone. Each syringe was also checked for air bubbles, and these were eliminated if present. All Exetainers® were then placed in a thermostatically controlled heater block set at 50^0C for 6 hours, and allowed to cool at room temperature for a minimum of 12 hours after reaction.

6.4 Analysis of Carbonate from Bio-apatite for Isotopic Analysis of ^{13}C and ^{18}O and Data Interpretation

In the human skeleton, oxygen is present in both the phosphate, and the carbonate fraction of bone apatite. This means that the ^{18}O composition of either fraction may be analysed, and used to calculate the likely δ^{18}O value of dietary water. In this research, the carbonate fraction was investigated because collagen is formed primarily from the protein portion of the diet, whereas bone carbonate represents the average of all dietary macronutrients (Ambrose and Norr, 1993). Sample analysis performed using an AP2003 Isotope Ratio Mass Spectrometer with gas sampling interface. It is essential when utilising the carbonate fraction to anchor the resulting δ^{18}O values on the VPDB scale, and then adjust them to the VSMOW scale on which $\delta^{18}O_{phosphate}$ values are traditionally reported (Paul *et al.*, 2007). This transference to a different scale is performed partly as a result of the equations designed to calculate the δ^{18}O value of dietary water. It is not a

single equation that is applied, but several designed to convert $\delta^{18}O_{carbonate}$ values into $\delta^{18}O_{phosphate}$ values, and $\delta^{18}O_{phosphate}$ values into $\delta^{18}O_{dietary\ water}$.

$\delta^{18}O_{VPDB}$ values of bone carbonate from a sample (x) can be converted into a $\delta^{18}O_{VSMOW}$ value by applying an equation (2) reported by Friedman and O'Neil in 1977 (Friedman and O'Neil, 1977):

$$\delta^{18}O_{carbonate\ VSMOW}(x) = 1.03086\ \delta^{18}O_{carbonate\ VPDB}(x) + 30.86 \qquad (2)$$

Once $\delta^{18}O_{carbonate}$ values have been transferred to the VSMOW scale they can be converted into $\delta^{18}O_{phosphate}$ values by employing an equation (3) produced by Iacumin and colleagues (1996):

$$\delta^{18}O_{phosphate} = 0.98\ \delta^{18}O_{carbonate} - 8.5 \qquad (3)$$

The original equation designed to convert $\delta^{18}O$ values of human bone phosphate to $\delta^{18}O$ values of source water (4) was developed by Longinelli (1984). However, the research utilised bone samples collected from individuals who died between the end of the 1800s and 1950. Acknowledging this data may not be suitable for comparison with a more modern population, Daux and colleagues (2008) reviewed the equation (5) using a contemporary sample. Considering the possible variations in both dietary intake and metabolism between these populations, the two equations are remarkably similar.

$$\delta^{18}O_{phosphate} = 0.64\ \delta^{18}O_{water} + 22.37 \qquad (4)$$

$$\delta^{18}O_{phosphate} = 0.65\ \delta^{18}O_{water} + 21.89 \qquad (5)$$

Daux and colleagues (2008) also investigated the impact of solid food consumption (in addition to that of drinking water) on the $\delta^{18}O$ values of skeletal phosphate, and developed equation (6). This study measured the oxygen composition of bone attributable to both drinking water and solid food water; therefore equation (6) shall be applied to estimate the $\delta^{18}O$ value of ingested water.

$$\delta^{18}O_{ingested\ water} = 1.54\ \delta^{18}O_{phosphate} - 33.72 \qquad (6)$$

Appendix 2 demonstrates the use of equations (2) – (6) for calculating the $\delta^{18}O$ value of ingested water from two $\delta^{18}O_{carbonate}$ values taken from the UoD and 792L bone sections.

6.5 Statistical Analysis

Raw data from the IRMS was transferred to Isodat; a software package designed to convert information for use in other software packages such as Microsoft Excel. The conversion of $\delta^{18}O_{carbonate}$ values into $\delta^{18}O_{phosphate}$ values, and $\delta^{18}O_{phosphate}$ values into $\delta^{18}O_{dietary\ water}$ was performed in Microsoft Excel. All statistical tests were performed using Sigmastat 3 and all graphs produced using Sigmaplot 10. Basic descriptive statistics were applied to all data to determine information such as means, standard errors, and standard deviations, and comparison of these means was performed using 2-way ANOVAs.

Descriptive statistical analyses were performed on oxygen and carbon data from all bone sections to acquire the means, standard deviations, and standard errors. A 2-way ANOVA was performed on the $\delta^{13}C$ and $\delta^{18}O$ values obtained from bone sections originating from the right and left femora of the same individual, with a view to determining the intra-individual variability. The same statistical test was applied to carbon and oxygen data collected from the holes drilled in one bone section, and a repeat analysis of the same sample to establish intra-sample variability. In addition, a 2-way ANOVA was performed on all ^{13}C and ^{18}O data originating from all drilled holes within the different bone sections. This test was employed to establish inter-individual variation in carbon and oxygen values.

The means of the likely $\delta^{18}O$ values of drinking water (see section 8.2) were compared with UK precipitation maps produced by Darling et al (2003) to estimate the geographical origin of the subjects. A 2-way ANOVA was performed on data collected from individuals of known provenance to establish whether the subjects (supposed to

have originated from the same region) could be distinguished from one another based on the likely $\delta^{18}O$ values of their drinking water. The same statistical test was applied to assess the difference between the $\delta^{13}C$ and $\delta^{18}O$ values from each drilled hole on the same bone section, in an attempt to determine intra-individual variability.

Chapter 7: Results

7.1 Results of Sample Analysis

Bone samples were collected both from cadavers used for the purposes of education from the University of Dundee, and from forensic cases. A total of three femoral sections have been analysed from the Dundee University cadavers, three forensic case samples have been analysed, and one femoral section was sourced from the University of Dundee teaching collection. Table 3 shows the basic information associated with the femoral sections. As can be seen from Table 3, there is very little available information on the sections arising from forensic casework. The specimens from the University of Dundee (apart from UoD, which was donated to the study from the teaching collection) all have an associated geographical history, age, sex, and cause of death (COD). The geographical history is defined as 'the last known location of the individual', for example this may have been a respite care home or hospital for the last few months or weeks of their life. The vast majority of femoral sections removed from cadavers at the University have originated from elderly individuals, this is due to the fact that most people decide to donate their bodies for education in the later stages of life. The samples 792L and 792R have both originated from the same individual, with 792L sampled from the left leg, and 792R from the right. The number of holes drilled in each bone section was dependent upon the width of the cortical bone. Some sections permitted sampling at two or even three sites in a line across the width of cortical bone (see Figure 24), whereas some of the thinner sections only allowed for the sampling of one site (see Figure 29).

Table 3. Table illustrating sample information. The forensic cases have no known history, whereas the majority of samples from the University of Dundee have an associated geographical history, age, sex, and cause of death.

Bone Section	Collection	Geographical History	Age	Sex	COD
JR3_14	Forensic casework	Unknown	Unknown	Unknown	Unknown
KAS2	Forensic casework	Unknown	Unknown	Unknown	Unknown
X65 D10	Forensic casework	Unknown	Unknown	Unknown	Unknown
UoD	University of Dundee teaching collection	Unknown	Unknown	Unknown	Unknown
792L & 792R	University of Dundee cadaver	Dundee	57	Male	End stage renal disease
820R	University of Dundee cadaver	Kirkcaldy	95	Female	chronic obstructive pulmonary disease and bronchopneumonia

The carbonate portion of the bone apatite was analysed for its carbon and oxygen isotopic content simultaneously. Basic descriptive statistical tests were run on all bone data to establish the means, and standard deviations. The raw data collected from analyses of all bone samples can be seen in Appendix 2. Table 4 shows the means, standard deviations for $\delta^{18}O$ values originating from all bone sections. The powdered sample collected from each drilled hole on all of the bone sections was analysed in triplicate. In some instances the amount of sample available for analysis in triplicate was insufficient (at least 15mg from each hole was required), and subsequently produced erroneous data. These have been omitted from statistical analyses (the reason why the total number of samples is not always divisible by the number of holes sampled). A repeat run of the KAS2 sample was analysed (labelled RPT) as there was enough sample available permitting the re-analysis of the collected samples at a later date. For all other bone sections, the amount of powdered sample collected from each of the holes was not enough to perform a repeat analysis on an alternative day. The mean $\delta^{18}O$ and $\delta^{13}C$ values for each hole taken from each section can be viewed in Appendices 10 to 22.

Table 4. Table describing the origin of the data, including the number of holes drilled, and number of samples. The $\delta^{18}O$ value and $\delta^{13}C$ value means of carbonate taken from all holes drilled in each bone section and their standard deviations are also reported.

Section	Number of holes sampled	Total number of samples	Mean $\delta^{18}O$ (‰) of bone carbonate	$\delta^{18}O$ Std Dev (‰)	Mean $\delta^{13}C$ (‰) of bone carbonate	$\delta^{13}C$ Std Dev (‰)
JR3_14	8	48	-5.13	0.95	-13.77	0.46
KAS2 and RPT	9 (x2)	96	-6.72	1.11	-16.66	0.85
X65 D10	15	36	-4.36	1.15	-14.71	0.65
UoD	12	48	-4.27	1.17	-15.67	0.23
792R	8	48	-2.83	0.91	-12.86	0.38
792L	8	48	-2.98	0.90	-14.82	4.50
820R	8	48	-2.92	1.49	-12.92	0.51

Table 4 shows the range for the $\delta^{18}O$ values for the bone sections to be 3.89‰ (-2.83 to -6.72‰). It also demonstrates that 792R had the highest $\delta^{18}O$ value of -2.83‰, and KAS2, the lowest with -6.72‰. The mean $\delta^{18}O$ value for 792L has also been highlighted as 792R and 792L originate from the same individual, and the two means of -2.98‰ for 792L and -2.83‰ for 792R are just 0.15‰ apart. However, the $\delta^{18}O$ mean values for these two sections are not as similar as that for 820R (-2.92‰) and 792L (-2.98‰) with a difference of just 0.06‰. This could be significant when attempting to determine whether the $\delta^{18}O$ values have originated from the same individual or different individuals. In light of this a one-way ANOVA was performed (see Appendix 4) to test the variation between the mean $\delta^{18}O$ values of 792L, 792R, and 820R. The p-value obtained for a comparison of the mean $\delta^{18}O$ values of 792L and 792R (originating from the same individual) was 0.700, indicating that there is not a statistically significant difference between the two means. A second one-way ANOVA was performed (Appendix 4), this time including 820R achieved a p-value of 0.802, suggesting that there is not a statistically significant difference between the mean $\delta^{18}O$ values obtained from bone sections 820R, 792L, and 792R.

The same information reported in Table 4 for $\delta^{18}O$ values, can also be seen for $\delta^{13}C$ values. The range of the $\delta^{13}C$ values for this dataset is 3.80‰ (-12.86 to -16.66‰), slightly less than that of the $\delta^{18}O$ values (3.89‰). As with the $\delta^{18}O$ values, KAS2 has the lowest $\delta^{13}C$ value of -16.66‰, and 792R the highest (-12.86‰). This suggests that there

is a relationship between the $\delta^{13}C$ and ^{18}O values, and this shall be explored further in this section. The $\delta^{13}C$ values between 792L and 792R (both highlighted) appear closely related (as with the $\delta^{18}O$ values), with a difference of just 0.053‰. A one-way ANOVA (see Appendix 5) confirms this relationship; with a p-value of 0.560 indicating that there is not a statistically significant difference between the $\delta^{13}C$ means of the two bone sections. The means of the $\delta^{13}C$ values originating from X65 D10 and 820R also appear similar, with the range between X65 D10 (-14.71‰) and 820R (-14.82‰) just 0.11‰. A one-way ANOVA (see Appendix 6) confirms the similarity between the values with a p-value of 0.887. It must however be noted that the standard deviation of 820R (4.50‰) is considerably higher than the nearest standard deviation value of 0.85‰ displayed by KAS2 and RPT (a difference of 3.65‰), 820R also has the lowest $\delta^{18}O$ value of -14.82‰ (see Table 4). The large standard deviation of 820R suggests that the sample was heterogeneous (mean $\delta^{13}C$ value -14.82‰). Closer inspection of the results from 820R indicates that this large standard deviation is likely to have resulted from a single hole, rather than contamination of the entire sample. Hole 6 has a standard deviation of 12.86‰, whereas all other holes have a standard deviation of between 0.1 and 0.3‰ (see Appendix 22) suggesting that hole 6 is the cause of sample hetrogeny. The p-value of 0.560 obtained from a comparison of mean $\delta^{13}C$ values between 792L and 792R (originating from the same individual), was less than that of the p-value from analysis of the mean $\delta^{13}C$ values of X65 D10 and 820R (0.887). This suggests that there is a less difference between the $\delta^{13}C$ values from X65 D10 and 820R even though they have not originated from the same individual (as 792L and 792R have).

Figure 19 shows a graph of the plotting of the mean $\delta^{18}O$ against the $\delta^{13}C$ mean values for all bone sections, with error bars. The graph demonstrates that visually, all samples can be distinguished from one another. The plots for the $\delta^{18}O$ and $\delta^{13}C$ value means for both 792L and 792R have been circled because they are remarkably close together. This is to be expected, as the sections have arisen from the left and right legs of the same individual. 820R clearly shows the most error for mean $\delta^{13}C$ values (around 10‰) and UoD (represented by the turquoise point) the least; while for oxygen data, 792L (represented by the green point) demonstrates the most variation, and 792R (represented

by the red dot, the least. Figure 19 demonstrates that the UoD and X65 D10 samples are very similar in oxygen values, as are samples 820R, 792L and 792R. Figure 19 also shows the carbon values for 820R and X65 D10 are similar, and this can be confirmed by Table 5. A visual analysis of error bars on all points indicates that the majority of samples show more variation in their mean $\delta^{18}O$ values than the associated $\delta^{13}C$ value means The only sample where the variation in mean $\delta^{13}C$ is greater than that of mean $\delta^{18}O$ for the same section is that of 820R. The regression analysis resulted in an R^2 value of 0.194 indicating that there is no correlation between the $\delta^{18}O$ and $\delta^{13}C$ values of samples.

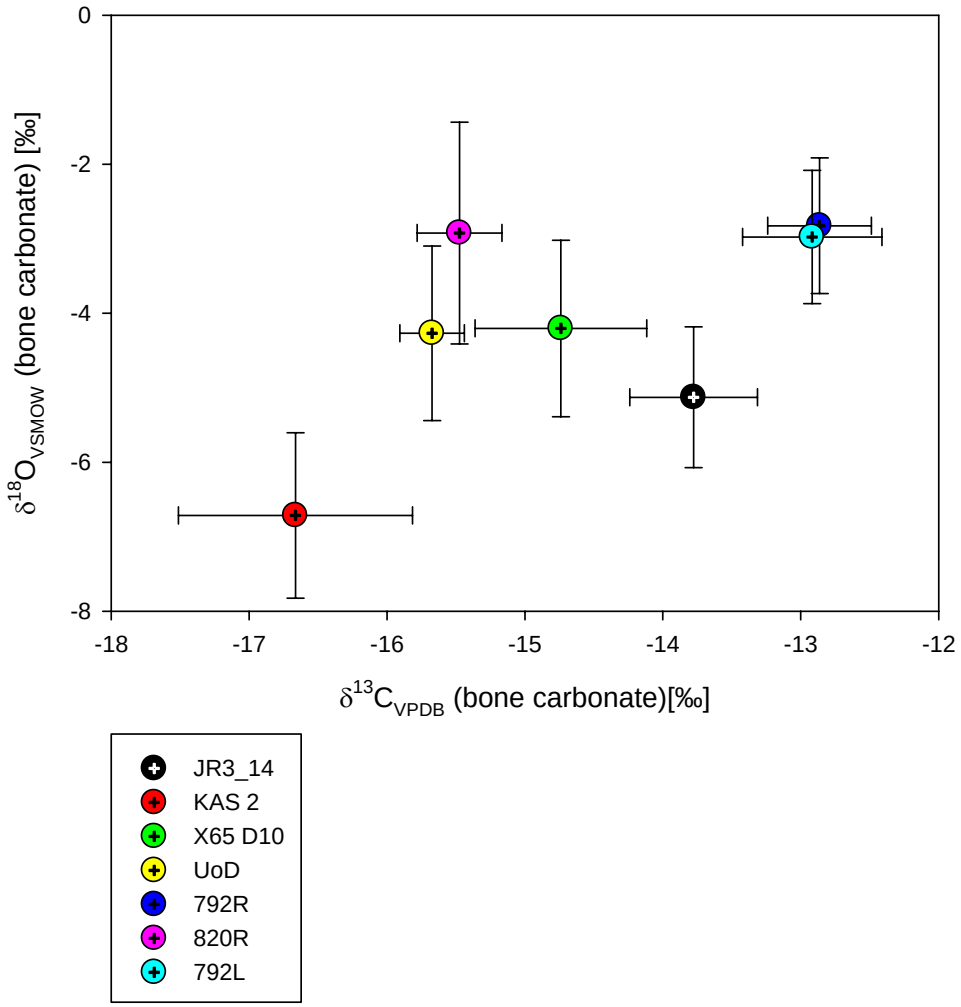

Figure 19. Graph showing the plots, with error bars, for $\delta^{18}O$ versus $\delta^{13}C$ mean values for all bone sections.

Table 6 shows the results of a one-way analysis of variance of the mean $\delta^{13}C$ values of all bone samples. The greatest difference is between the $\delta^{13}C$ of 792R and KAS2 with a difference between the means of 3.80‰, with the ANOVA confirming a statistically significant difference (p-value <0.001). There is also a statistically significant difference between the $\delta^{13}C$ mean values for 792L and Kas2, JR3_14 and Kas2, 792R and UoD, 792L and UoD, 820R and KAS2, X65 D10 and Kas2, 792R and 820R, JR3_14 and UoD, 792R and X65 D10, 792L and X65 D10, UoD and Kas, JR3_14 and 820R, and 820R and UoD. 820R and UoD have the least difference between their means (0.85‰) of those sections with a statistically significant difference between their $\delta^{13}C$ values. Table 6 also demonstrates that the $\delta^{13}C$ values of some of the compared sections are not significantly different. When the $\delta^{13}C$ values were compared, 792R and JR_3 14, X65 D10 and UoD, JR_3 14 and X65 D10, 792L and JR_3 14, X65 D10 and 820R, and 792R and 792L did not show a significant difference. In particular the difference of the means between 792R and 792L (0.05‰) and between X65 D10 and 820R (0.11‰) were quite similar. The least difference in $\delta^{13}C$ values was that between 792R and 792L with a p-value of 0.879. This is justifiable, as both sections originated from the same individual, and would be expected to have very similar isotopic compositions.

Table 5. The results of a one-way ANOVA, run to compare the means of $\delta^{13}C$ values from all bone sections. The majority of sections demonstrate a significant difference between their mean $\delta^{13}C$ values.

Bone Sections for Comparison of Mean $\delta^{13}C$ Values	Difference of Means (‰)	Unadjusted P	Significant?
792R vs. KAS2	3.80	<0.001	Yes
792L vs. KAS2	3.75	<0.001	Yes
JR3_14 vs. KAS2	2.89	<0.001	Yes
792R vs. UoD	2.81	<0.001	Yes
792L vs. UoD	2.76	<0.001	Yes
820R vs. KAS2	1.84	<0.001	Yes
X65 D10 vs. KAS2	1.95	<0.001	Yes
792R vs. 820R	1.96	<0.001	Yes
792L vs. 820R	1.91	<0.001	Yes
JR3_14 vs. UoD	1.90	<0.001	Yes
792R vs. X65 D10	1.85	<0.001	Yes
792L vs. X65 D10	1.80	<0.001	Yes
UoD vs. KAS2	0.99	0.001	Yes
JR3_14 vs. 820R	1.05	0.003	Yes
792R vs. JR3_14	0.91	0.009	No
X65 D10 vs. UoD	0.96	0.011	No
JR3_14 vs. X65 D10	0.94	0.013	No
792L vs. JR3_14	0.86	0.014	No
820R vs. UoD	0.85	0.015	Yes
X65 D10 vs. 820R	0.11	0.774	No
792R vs. 792L	0.05	0.879	No

Table 7 shows the results of a one-way analysis of variance of the mean $\delta^{18}O$ values of all bone samples. The greatest difference is between the oxygen content of 792R and KAS2 with a difference between the means of 3.89‰, with the ANOVA confirming a statistically significant difference (p-value <0.001). These two bone sections also had the greatest difference between their carbon values (Table 6).There is also a statistically significant difference between the $\delta^{18}O$ mean values for 792L and Kas2, 820R and Kas2, UoD and KAS2, X65 D10 and KAS2, 792R and JR_3 14, 792L and JR_3 14, 820R and JR_3 14, JR_3 14 and KAS2, 792R and UoD, 792R and X65 D10, 792L and UoD, 792L and X65 D10, 820R and UoD, 820R and X65 D10, UoD and JR_3 14, X65 D10 and JR_3 14, and 792R and 820R.. 792R and 820R have the least difference between their means (0.15‰) of those sections with a statistically significant difference between their $\delta^{18}O$ values. Table 7 also demonstrates that the $\delta^{18}O$ values of some of the compared sections are not significantly different. When the $\delta^{18}O$ values were compared, 792R and

792L, UoD and X65 D10, and 792L and 820R did not show a significant difference. In particular the difference of the means between 792L and 820R (0.05‰) were quite similar. The least difference in $\delta^{18}O$ values was that between 792L and 820R with a p-value of 0.811. This shows that there is less difference between the means of 792L and 820R (0.05‰) which have originated from different individuals, than 792L and 792R (0.10‰) are both from the same individual.

Table 6. The results of a one-way ANOVA, run to compare the means of $\delta^{18}O$ values from all bone sections

Bone Sections for Comparison of Means of $\delta^{18}O$ values	Difference of Means (‰)	Unadjusted P	Significant?
792R vs. KAS2	3.89	<0.001	Yes
792L vs. KAS2	3.79	<0.001	Yes
820R vs. KAS2	3.74	<0.001	Yes
UoD vs. KAS2	2.45	<0.001	Yes
X65 D10 vs. KAS2	2.36	<0.001	Yes
792R vs. JR3_14	2.30	<0.001	Yes
792L vs. JR3_14	2.21	<0.001	Yes
820R vs. JR3_14	2.15	<0.001	Yes
JR3_14 vs. KAS2	1.59	<0.001	Yes
792R vs. UoD	1.44	<0.001	Yes
792R vs. X65 D10	1.53	<0.001	Yes
792L vs. UoD	1.35	<0.001	Yes
792L vs. X65 D10	1.43	<0.001	Yes
820R vs. UoD	1.29	<0.001	Yes
820R vs. X65 D10	1.38	<0.001	Yes
UoD vs. JR3_14	0.86	<0.001	Yes
X65 D10 vs. JR3_14	0.77	0.002	Yes
792R vs. 820R	0.15	0.504	Yes
792R vs. 792L	0.10	0.667	No
UoD vs. X65 D10	0.09	0.722	No
792L vs. 820R	0.05	0.811	No

As discussed previously, the samples KAS2 and RPT are taken from one bone section. KAS2 was initially sampled, and the powdered bone collected from each drilled hole and analysed. There was enough sample remaining to repeat this analysis, resulting in the RPT sample. Up until this point, KAS2 and RPT have been analysed as the same sample, as they originate from the same individual. Considering these two datasets were collected from the same section, the same drilled holes, and even the same powdered sample from these holes, it is likely both the $\delta^{18}O$ and $\delta^{13}C$ values will show similar results. Table 8 shows the mean $\delta^{13}C$ values to be similar, with a range of 0.64‰ (-16.37 to -16.98). RPT

has a slightly lower $\delta^{13}C$ value of -16.98‰ than that of KAS2 at -16.34‰. A one-way ANOVA was performed to statistically assess this variation (see Appendix 7). The resulting p-value of <0.001 suggests a significant statistical difference between the $\delta^{13}C$ values for KAS2 and RPT. Statistical analysis of the standards run with the samples (see Appendix 8) demonstrates that the majority of variation between the two samples is likely to have arisen during preparation and storage. Appendix 8 shows the standard deviations for NBS-19, Bicarb-X and LSVEC to be small (all under 0.8), and lower than that of the standard deviation for KAS2 (1.01‰). These standards were stored and prepared by the lab technician at the SCRI Stable Isotope Facility.

Table 7. Table reporting the mean and standard deviation $\delta^{`13}C$ values of samples KAS2 and RPT

Section	N	Missing	Mean $\delta^{13}C$ (‰)	Std Dev (‰)
KAS2	48	0	-16.34	1.01
RPT	48	0	-16.98	0.48

Table 9 reports the mean $\delta^{18}O$ values for both KAS2 and RPT. As can be seen in this table the mean $\delta^{18}O$ values are very similar, with the range being 0.28‰; greater than that of the $\delta^{13}C$ values. The lower of the two figures comes from RPT at -6.87‰, and the higher from KAS2 at -6.56‰. A one way ANOVA resulted in a p-value of 0.167 (see Appendix 7), suggesting there is not a statistically significant difference between the $\delta^{18}O$ values obtained from KAS2 and RPT.

Table 8. Table reporting the mean and standard deviation $\delta^{18}O$ values of samples KAS2 and RPT

Section	N	Missing	Mean $\delta^{18}O$ (‰)	Std Dev (‰)
KAS2	48	0	-6.56	1.31
RPT	48	0	-6.87	0.85

The mean $\delta^{18}O$ values from the bone sections of known origin were used to estimate the oxygen composition of the individual's drinking water (see Appendix 2), the equations for which can be viewed in Chapter 7 (Method and Materials). The samples of known origin are 820R (Kirkcaldy) and 792L and 792R (both Dundee), all three of which have 8 holes drilled in them. Figure 20 shows a plot of these likely oxygen values, with error

bars for each section. The mean calculated $\delta^{18}O$ values for dietary water are -7.79‰ for 820R, -7.88‰ for 792L, and -6.35‰ for 792R (the full results can be seen in Appendix 9). This is not as expected, as femoral sections 792L and 792R have originated from the same individual, and would therefore be expected to have more similar $\delta^{18}O$ drinking water values than 792L and 820R. The error bars indicate that the largest spread of data from a single bone sample is that of 820R (represented by the orange dot) of around 2‰. Statistical comparison of the estimated $\delta^{18}O$ dietary water values with the average $\delta^{18}O$ value calculated from 62 water samples collected in Dundee (see Appendix 23) demonstrates that there is not a statistically significant difference (p value = 1).

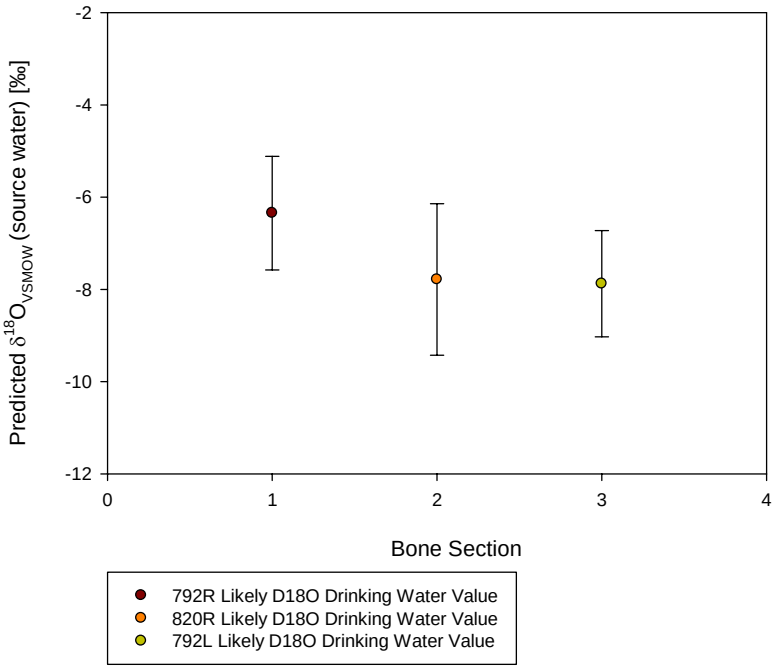

Figure 20. Graph showing the plots, with error bars, for predicted $\delta^{18}O$ values of dietary water for bone sections 792L, 792R, and 820R.

In an attempt to assess intra-individual variability, several samples were taken from each femoral section, by drilling holes in locations around the bone (see Figure 18, Chapter 6). Each drilled hole was then assigned a number, and the powdered sample from each hole was analysed for both $\delta^{13}C$ and $\delta^{18}O$ composition. One-way ANOVAs were performed

on all data, with a view to establishing whether there is a significant difference in the isotopic values obtained from the same individual.

The results from the descriptive statistical tests applied to $\delta^{13}C$ and $\delta^{18}O$ values collected from sample JR_3 14 are shown in Appendix 9. Hole number 8 had the lowest mean $\delta^{13}C$ value at -14.54‰. Hole number 5 had the highest mean $\delta^{13}C$ value (-13.10‰), with the difference between these values being 1.44‰. Hole number 8 also had the greatest standard deviation at 0.39‰. The highest mean $\delta^{18}O$ value was obtained from hole number 1 (-3.87‰), and the lowest from number 7 (-5.87‰), with a range of 2.01‰; greater than that for the carbon values. The graph in Figure 21 demonstrating the results of the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the greatest amount of variation is on the y-axis (the $\delta^{18}O$ values), with the range of data from -6.7‰ to -3‰ (a difference of 3.7‰). On the x-axis, the data is grouped from around -14.9‰ to -12.7‰ (a range of 2.2‰). This suggests the $\delta^{18}O$ data for JR_3 14 is more variable than the δ13C values. Figure 21 also presents the close relationship between the isotopic composition of hole 1 and hole 2, and in addition illustrates that the carbon values of holes 1, 2 and 3 are extremely similar as are the oxygen values for holes 5, 7, and 8. One-way ANOVAs were performed to test the variation between both mean $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 10. The p-value (<0.001) of the $\delta^{13}C$ ANOVA suggests there is a statistically significant difference between the $\delta^{13}C$ values obtained from the holes drilled in JR_3 14. A p-value of <0.001 for the ANOVA performed on $\delta^{18}O$ data also suggests there is a statistically significant difference between the $\delta^{18}O$ values measured from the samples collected from JR_3 14.

Figure 22 illustrates the location of the holes drilled on the JR_3 14. As previously mentioned, holes 1 and 2 have a close relationship when carbon and oxygen values are plotted against each other. The locations of the two sampling sites are also in very close proximity when examined in Figure 22. Holes 5, 7 and 8 also share a close proximity, and have been collected from the same side of the bone section. The graph in Figure 21 also shows that holes 1 and 8 are the furthest from each other in mean $\delta^{13}C$ and $\delta^{18}O$ values, which corresponds to the schematic in Figure 22.

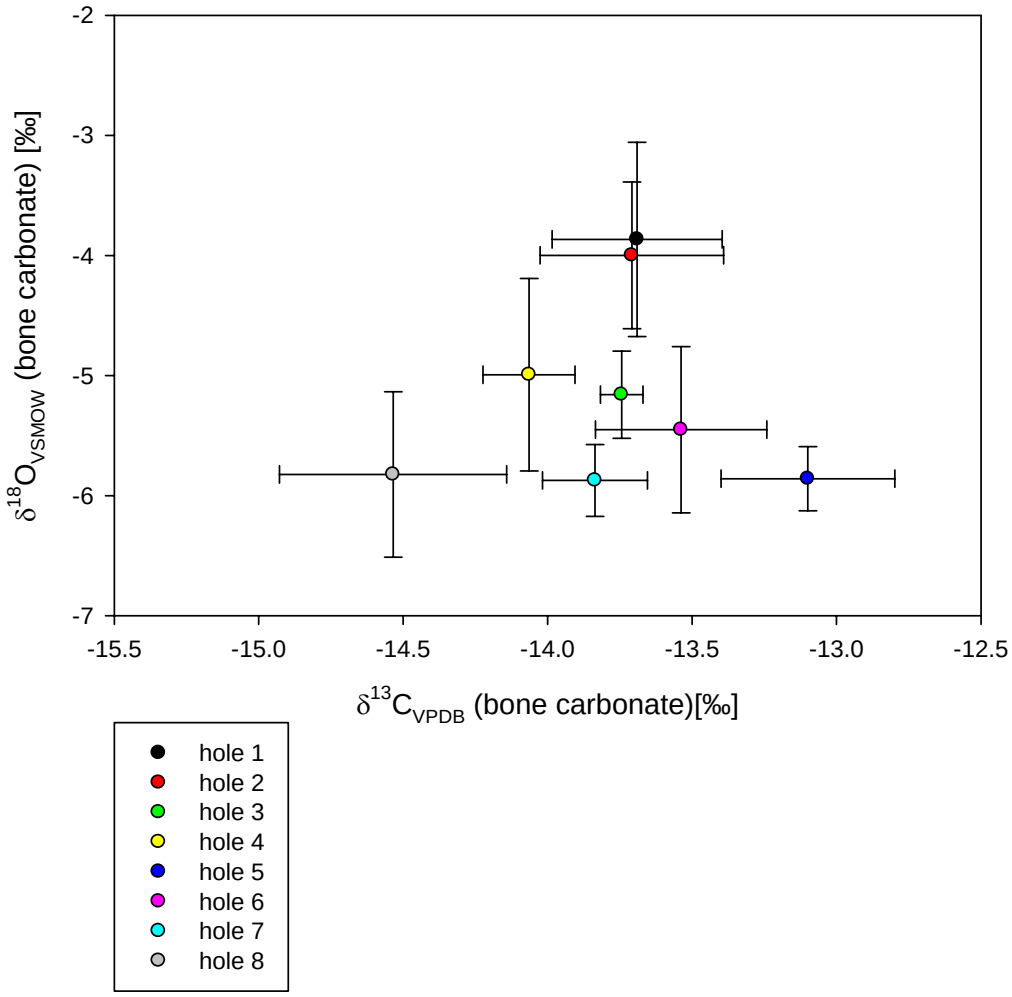

Figure 21. Graph showing the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values, with error bars, for individual samples of JR_3 14.

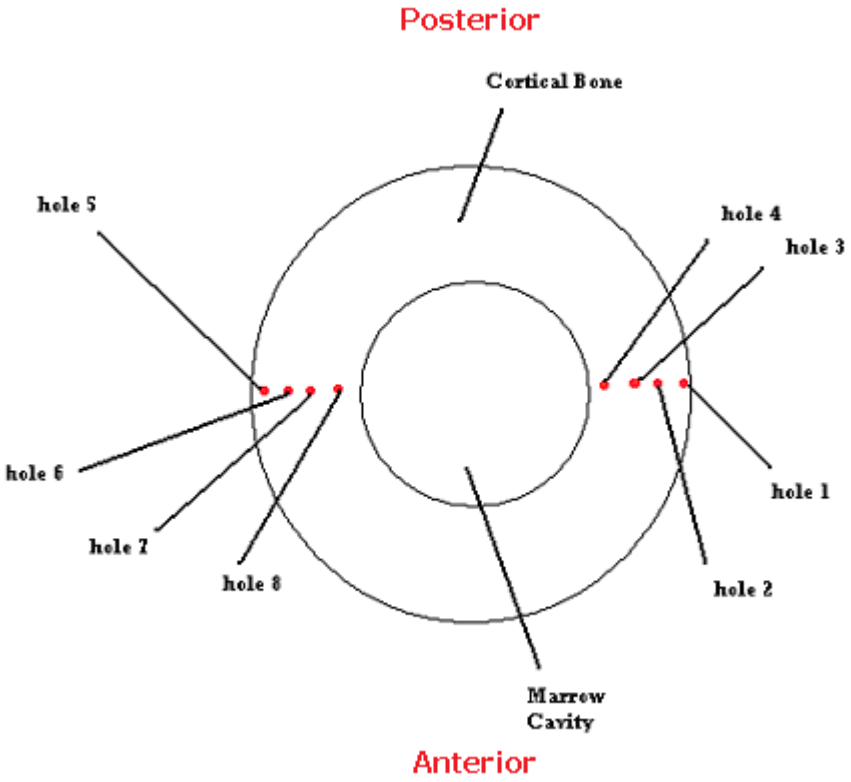

Figure 22. Schematic illustrating the locations of drilled holes from JR_3 14.

The results from the descriptive statistical tests applied to $\delta^{13}C$ and $\delta^{18}O$ values collected from sample X65 D10 are shown in Appendix 11. Hole number 1 had the lowest mean $\delta^{13}C$ value at -15.29‰. Hole number 18 had the highest mean $\delta^{13}C$ value (-14.18‰), with the difference between these $\delta^{13}C$ values being 1.11‰. The highest mean $\delta^{18}O$ value was obtained from hole number 7 (0.49‰), and the lowest from number 6 (-7.03‰), with a range of 6.53‰; substantially greater than that for mean carbon values. Figure 23 demonstrates the similar carbon and oxygen values of hole 10 and hole 14, and hole 1 and hole 4. Figure 24 demonstrates that there is no relationship between the proximity of these holes, and their similarity in carbon and oxygen values. The graph in Figure 23 demonstrating the results of the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the greatest amount of variation is on the y-axis, with the range of data from around -7‰ to 1‰ (a difference of 8‰). On the x-axis, the data is grouped from -16.3‰ to -13.6‰ (a range of 2.7‰). The graph also presents the close relationship between the isotopic composition of hole 10 and hole 14, and hole 4 and hole 16. Analysis of the schematic shown in Figure 24 does not show these sampling sites to be in close proximity

to each other. One-way ANOVAs were performed to test the variation between both mean $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 12. The p-value (<0.001) of the $\delta^{13}C$ ANOVA suggests there is a statistically significant difference between the mean $\delta^{13}C$ values obtained from the holes drilled in X65 D10. A p-value of <0.001 for the ANOVA performed on mean $\delta^{18}O$ data also suggests there is a statistically significant difference between the $\delta^{18}O$ mean values measured from the samples collected from X65 D10.

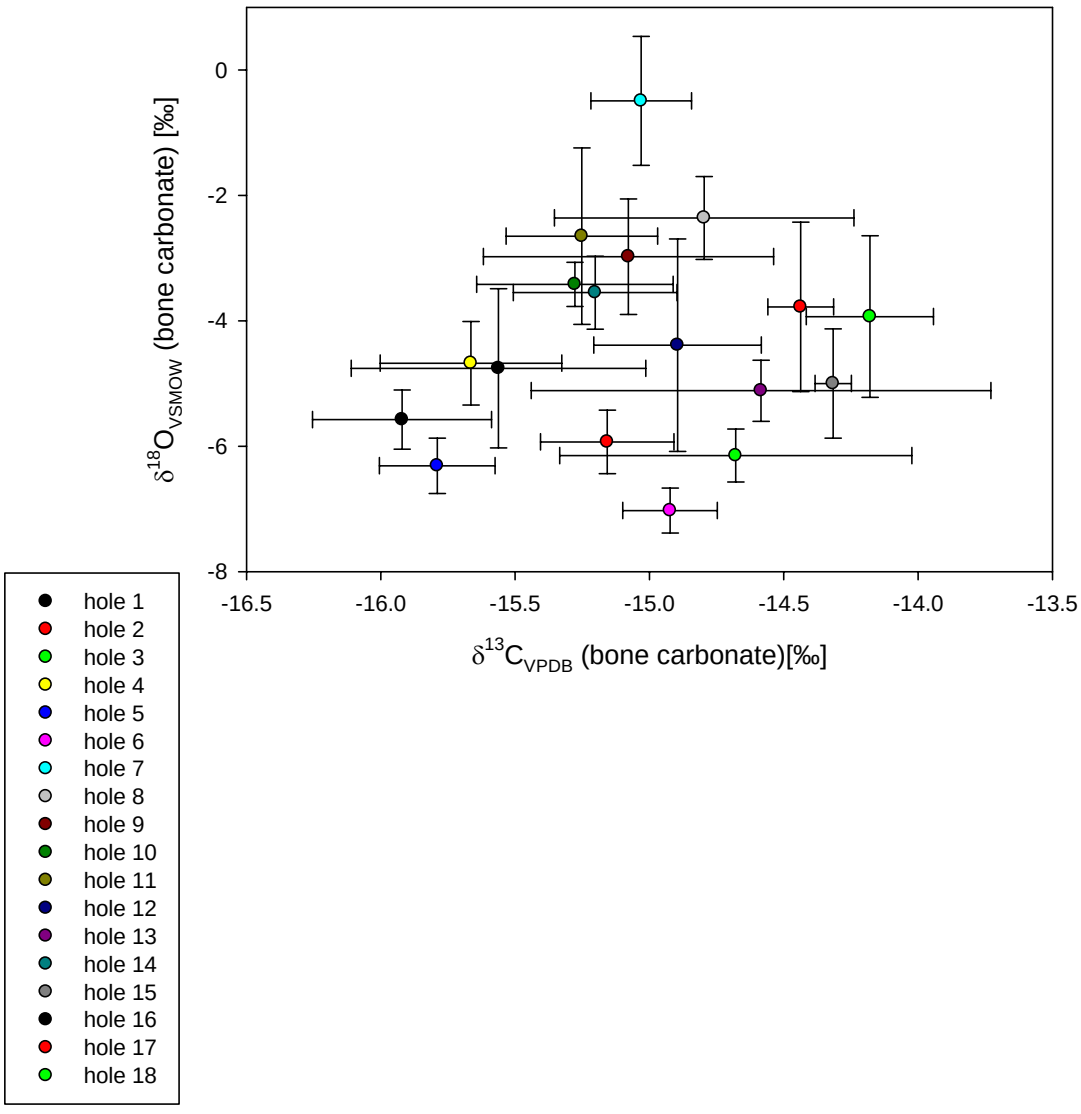

Figure 23. Graph showing the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values, with error bars, for individual samples of X65 D10.

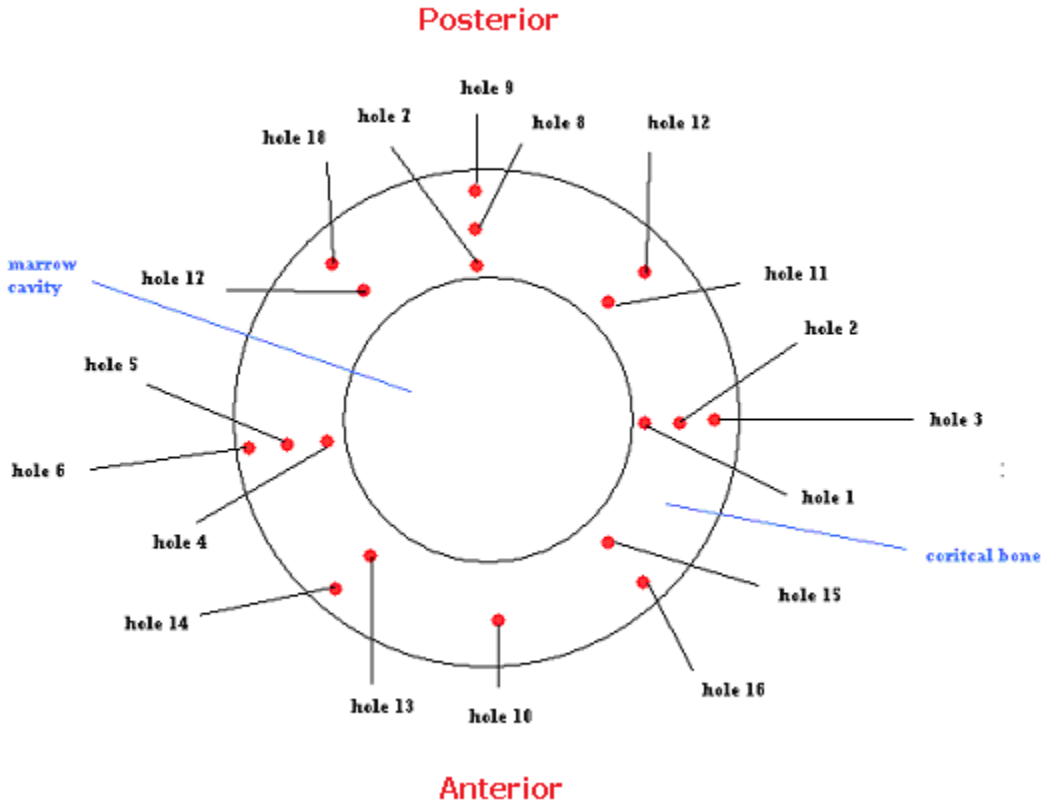

Figure 24. Schematic illustrating the locations of drilled holes from X65 D10

The data obtained from descriptive statistical analysis of $\delta^{13}C$ and $\delta^{18}O$ values from section KAS2 (and RPT) can be viewed in Appendix 13. Hole number 3 had the lowest mean $\delta^{13}C$ value at -17.22‰. Hole number 1 had the highest mean $\delta^{13}C$ value (-15.77‰), with the difference between these $\delta^{13}C$ values being 1.49‰. The highest mean $\delta^{18}O$ value was obtained from hole number 1 (4.68‰), and the lowest from number 6 (-7.03‰), with a range of 2.64‰; slightly more than for mean $\delta^{13}C$ values. The graph in Figure 25 demonstrating the results of the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the majority of data is closely grouped, apart from one datapoint, that of hole 1. Hole 1 also has the largest error bars of all datapoints plotted. For this reason a second graph was produced (see Figure 26), excluding the results collected from hole 1. Figure 26 demonstrates that the closest isotopic values are that of samples from hole 6 and hole 9, with the next closest relationship that of hole 8 and hole 2. It also illustrates the

similarities in oxygen values of holes 4 and 8, and holes 6 and 7. In addition, Figure 26 shows the carbon isotope measurements for holes 3 and 4 to be close in value. A visual assessment of the schematic in Figure 27 showing the region of sampling sites demonstrates that there is no location-based similarity between holes 6 and 9, and 8 and 2 respectively. One-way ANOVAs were performed to test the variation between both $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 14. The p-value (<0.001) of the mean $\delta^{13}C$ ANOVA suggests there is a statistically significant difference between the $\delta^{13}C$ mean values obtained from the holes drilled in KAS2 (and RPT). A p-value of <0.001 for the ANOVA performed on $\delta^{18}O$ mean data also suggests there is a statistically significant difference between the mean $\delta^{18}O$ values

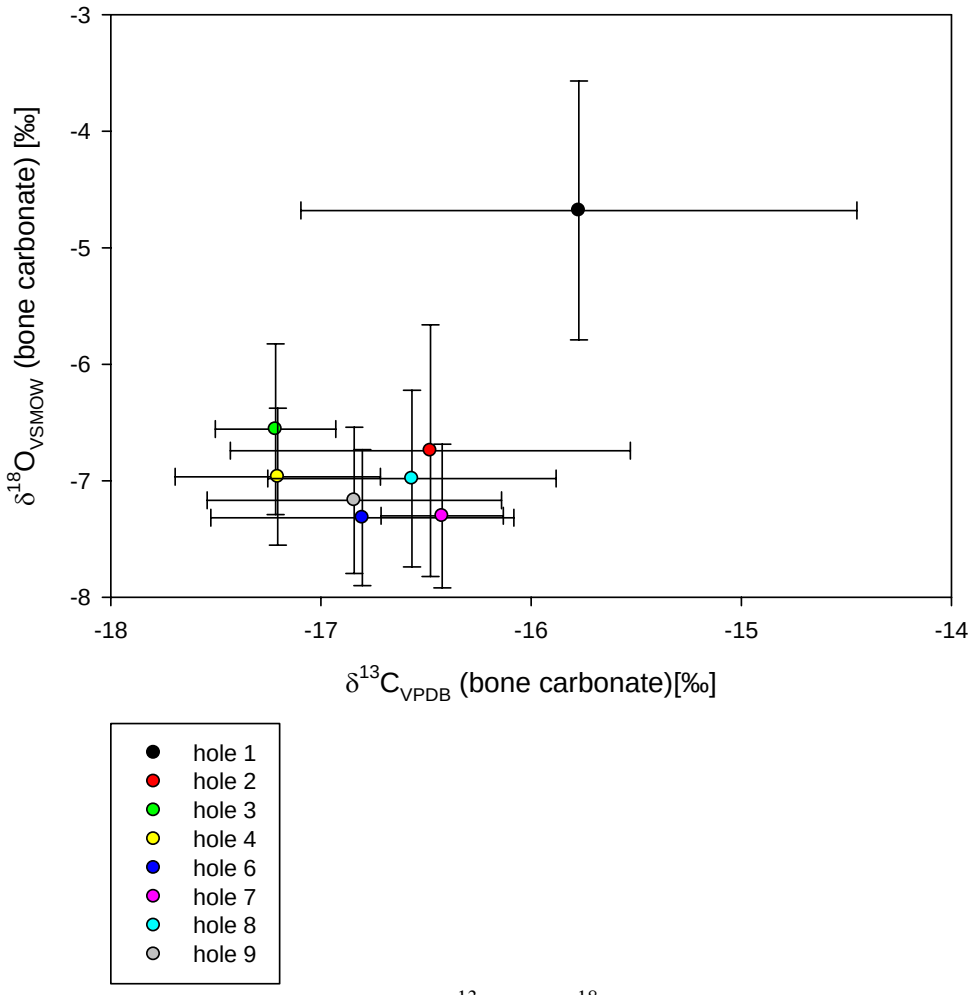

Figure 25. Graph showing the plots of $\delta^{13}C$ and $\delta^{18}O$ mean values, with error bars, for individual samples of KAS2 and RPT.

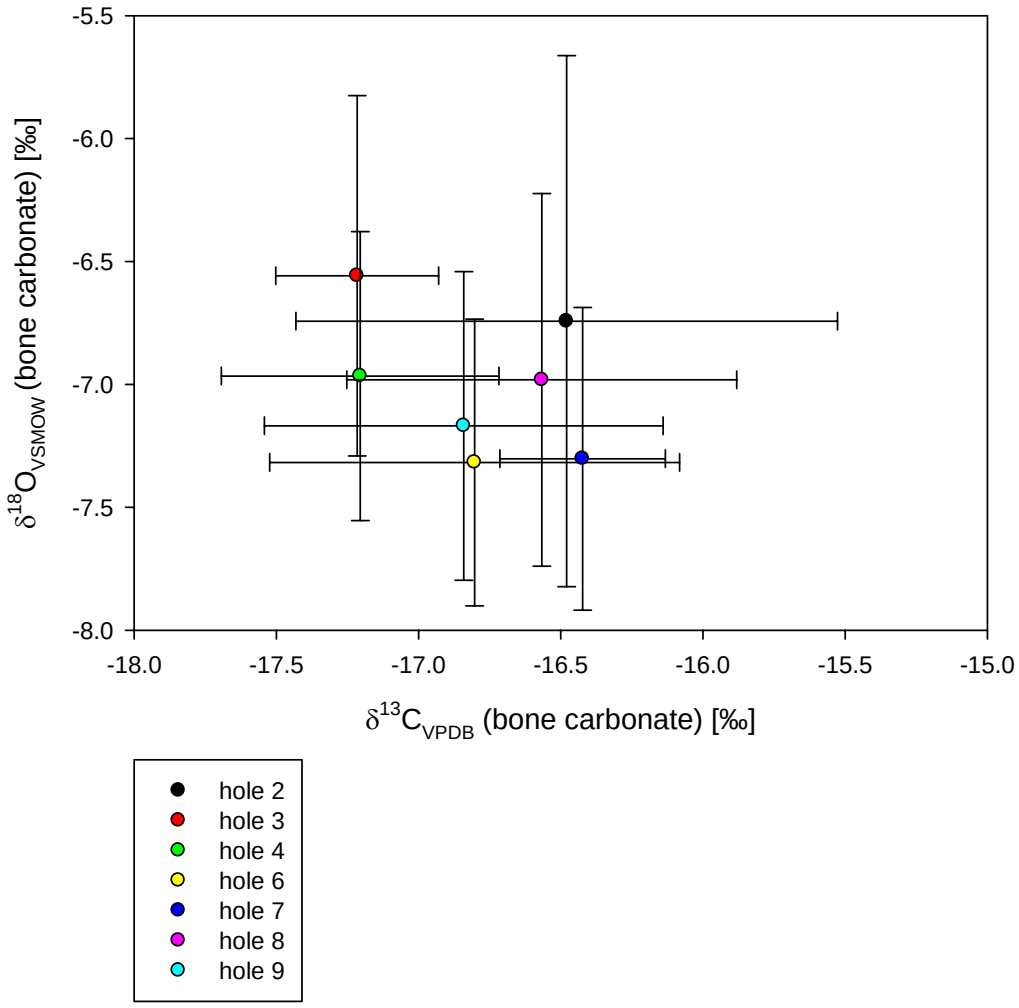

Figure 26. Graph showing the plots of $\delta^{13}C$ and $\delta^{18}O$ mean values, with error bars, for individual samples of KAS2 and RPT excluding hole number 1.

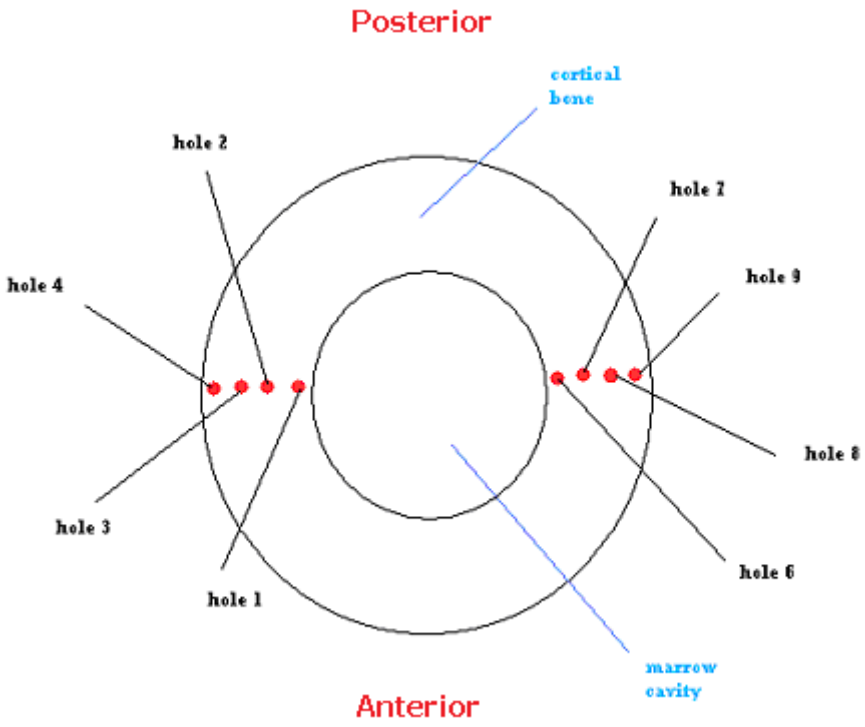

Figure 27. Schematic illustrating the locations of drilled holes from KAS2 and RPT.

The results from the descriptive statistical tests applied to $\delta^{13}C$ and $\delta^{18}O$ values collected from sample 792L are shown in Appendix 15. Hole number 4 had the lowest mean $\delta^{13}C$ value at -13.49‰. Hole number 1 had the highest mean $\delta^{13}C$ value (-12.36‰), with the difference between these $\delta^{13}C$ values being 1.11‰. The highest mean $\delta^{18}O$ value was obtained from hole number 4 (-2.37‰), and the lowest from number 8 (-3.81‰), with a range of 1.44‰; only 0.33‰ higher than that for mean carbon values. The graph in Figure 28 demonstrating the results of the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the greatest amount of variation is in $\delta^{18}O$ values (y-axis), with the range of data from around -4.5‰ to -1.3‰ (a difference of 3.2‰). On the x-axis, the data is spread from around -13.6‰ to -12‰ (a range of 1.6‰). Figure 28 also shows that hole 5 (blue point) has the greatest variation in $\delta^{13}C$ values, and for $\delta^{18}O$ values holes 1, 5, and 7 have the largest variation. In addition, the graph demonstrates the close relationship between the isotopic composition of hole 2 and hole 7 for both carbon and oxygen values, and hole 6 and hole 3 particularly for oxygen values. Holes 4 and 6 are

very similar in carbon values, as are holes 5 and 9. It can also be seen that the oxygen values for holes 3, 5, and 6 are similar, as are those of holes 1, 2, and 7. Figure 29 is an illustration of the locations of sampling sites on 792L, and suggests there is no correlation between the area the sample was collected from, and similarities in $\delta^{13}C$ and $\delta^{18}O$ mean values. One-way ANOVAs were performed to assess variation between both mean $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 16. The p-value (<0.001) of the $\delta^{13}C$ ANOVA suggests there is a statistically significant difference between the mean $\delta^{13}C$ values obtained from the holes drilled in 792L. A p-value of 0.079 for the ANOVA performed on $\delta^{18}O$ data indicates there is no significant difference between the $\delta^{18}O$ mean values measured from the samples collected from 792L.

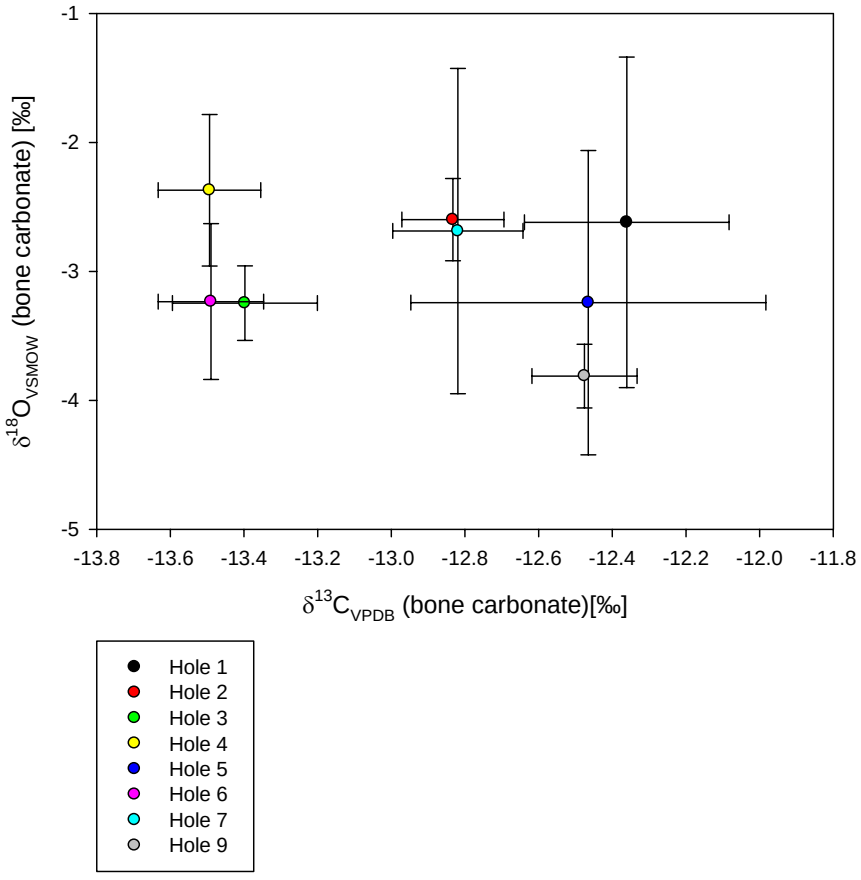

Figure 28. Graph showing the plots of $\delta^{13}C$ and $\delta^{18}O$ mean values, with error bars, for individual samples of 792L.

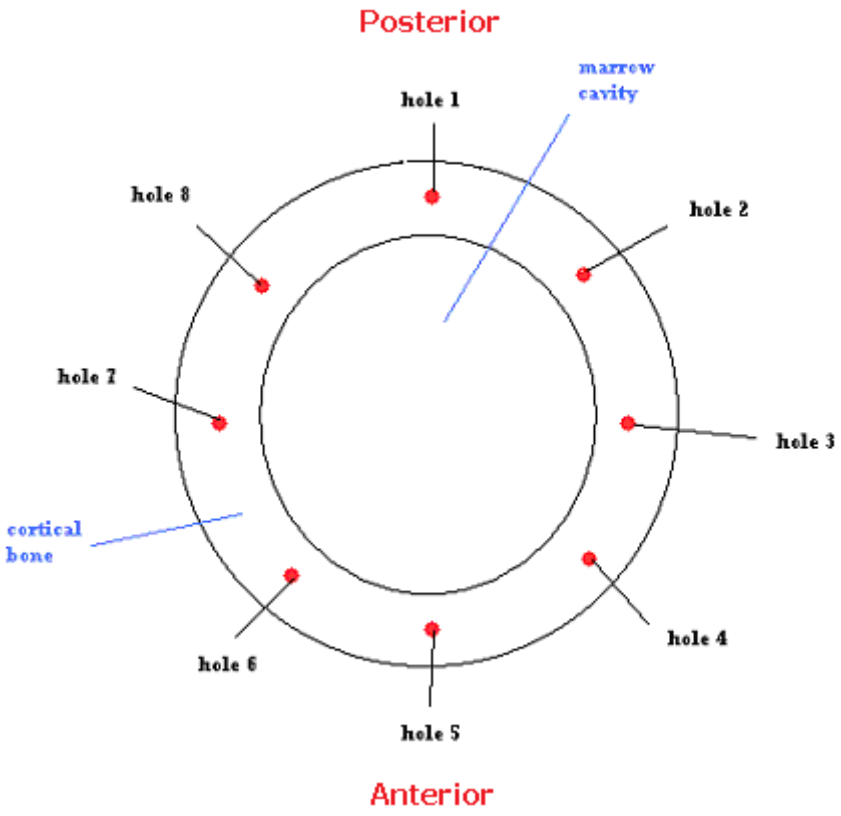

Figure 29. Schematic illustrating the locations of drilled holes from 792L

The results from the descriptive statistical tests applied to $\delta^{13}C$ and $\delta^{18}O$ values collected from sample 792R are shown in Appendix 17. Hole number 8 has the lowest mean $\delta^{13}C$ value at -13.10‰. Hole number 7 had the highest mean $\delta^{13}C$ value (-12.70‰), with the difference between these $\delta^{13}C$ values being very slight at 0.40‰. The highest mean $\delta^{18}O$ value was obtained from hole number 1 (-1.64‰), and the lowest from number 8 (-4.19‰), with a range of 2.55‰; substantially greater than that for the carbon values. The graph in Figure 30 demonstrating the results of the plots of $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the greatest amount of variation is on the y-axis (the $\delta^{18}O$ values), with the range of data from around -0.6‰ to -4.4‰ (a difference of -3.8‰). On the x-axis, the data is grouped from around -12.1‰ to -13.5‰ (a range of 1.4‰). This suggests the $\delta^{18}O$ data for 792R is more variable than the δ13C values. Figure 30 demonstrates that holes 1, 2, 3, and 6 have similar carbon values, and holes 4, 5, and 6 similar oxygen values. The graph also presents the close relationship between hole 6,

hole 2, hole 3, and hole 5 in terms of both carbon and oxygen values. This can be investigated further by studying the illustration in Figure 31. Hole 2 and 3 are in close proximity to each other, as are 5 and 6. The most similar $\delta^{13}C$ and $\delta^{18}O$ values suggested by Figure 30 were however those of hole 2 and hole 6, which are opposite each other on the illustration. One-way ANOVAs were performed to test the variation between both mean $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 18. The p-value (0.652) of the $\delta^{13}C$ ANOVA suggests there is not a significant difference between the $\delta^{13}C$ mean values obtained from the holes drilled in 792R. A p-value of <0.001 for the ANOVA performed on mean $\delta^{18}O$ data suggests there is a statistically significant difference between the mean $\delta^{18}O$ values measured from the samples collected from 792R.

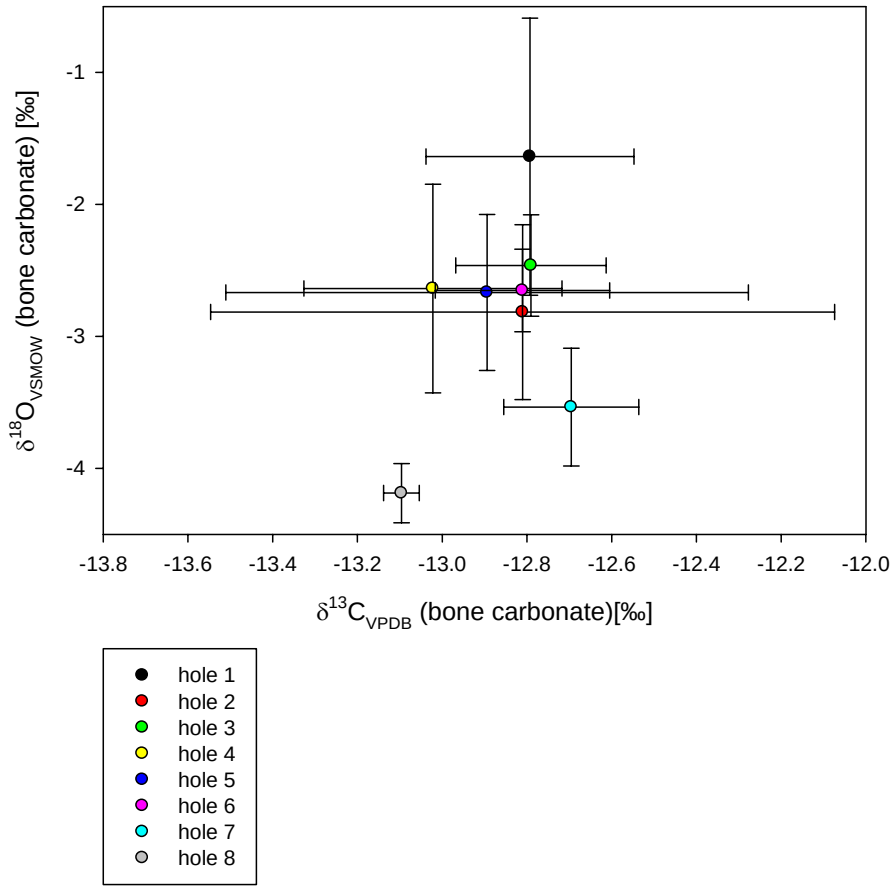

Figure 30. Graph showing the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values, with error bars, for individual samples of 792R

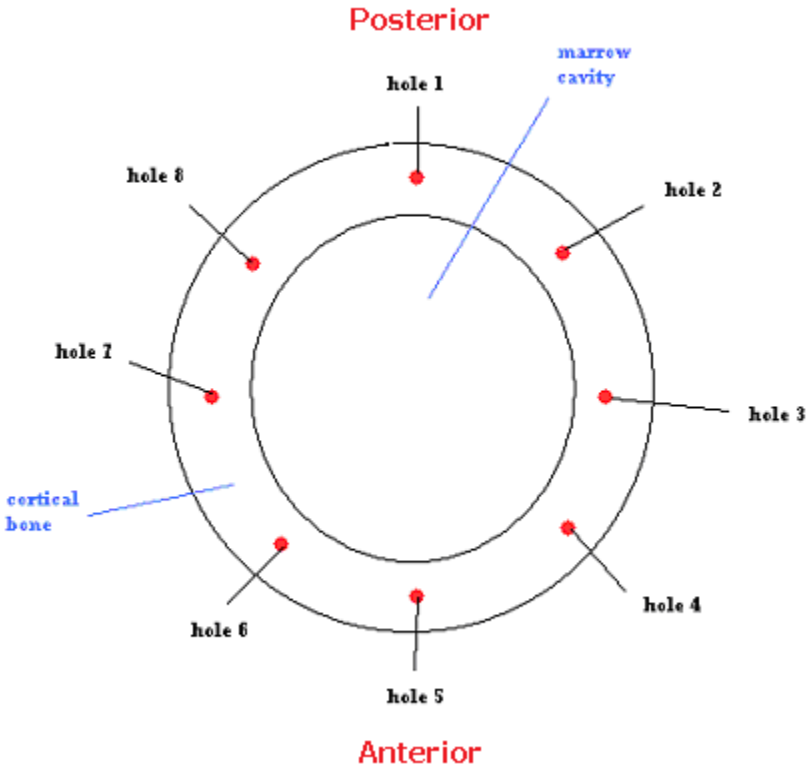

Figure 31. Schematic illustrating the locations of drilled holes from 792R

The data obtained from descriptive statistical analysis of $\delta^{13}C$ and $\delta^{18}O$ values obtained from section UoD can be viewed in Appendix 19. Hole number 15 had the lowest mean $\delta^{13}C$ value at -16.06‰. Hole number 5 had the highest mean $\delta^{13}C$ value (-15.49‰), with the difference between these $\delta^{13}C$ values being very low at 0.56‰. The highest mean $\delta^{18}O$ value was obtained from hole number 1 (1.919‰), and the lowest from number 7 (-5.68‰), with a range of 3.77‰; significantly more than for $\delta^{13}C$ values. The graph in Figure 32 demonstrating the results of the plots of $\delta^{13}C$ and $\delta^{18}O$ values for each hole shows visually that the greatest amount of variation is in $\delta^{18}O$ values (y-axis), with the range of data from around -6‰ to -1.5‰ (a difference of 4.5‰). On the x-axis, the data is spread from around -15.1‰ to -16.1‰ (a range of 1‰). The isotopic composition of hole 1 appears to be quite different to that of the others, as its datapoint is located away from the main cluster (see Figure 32). The closest group of points for carbon values is that of hole 12, hole 9, and hole 14. For oxygen values it appears to be holes 5, 8 and hole

13. Investigation of the illustration in Figure 33 shows that holes 12 and 9 are opposite each other, but there is no clear relationship between 14 and 9 or 14 and 12. There is also no relationship between the locations of holes 5, 8, and 13. One-way ANOVAs were performed to test the variation between both mean $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 20. The p-value (0.160) of the $\delta^{13}C$ ANOVA suggests there is not a significant difference between the $\delta^{13}C$ values obtained from the holes drilled in UoD. A p-value of <0.001 for the ANOVA performed on $\delta^{18}O$ mean data suggests there is a statistically significant difference between the mean $\delta^{18}O$ values collected from sampling sites on UoD.

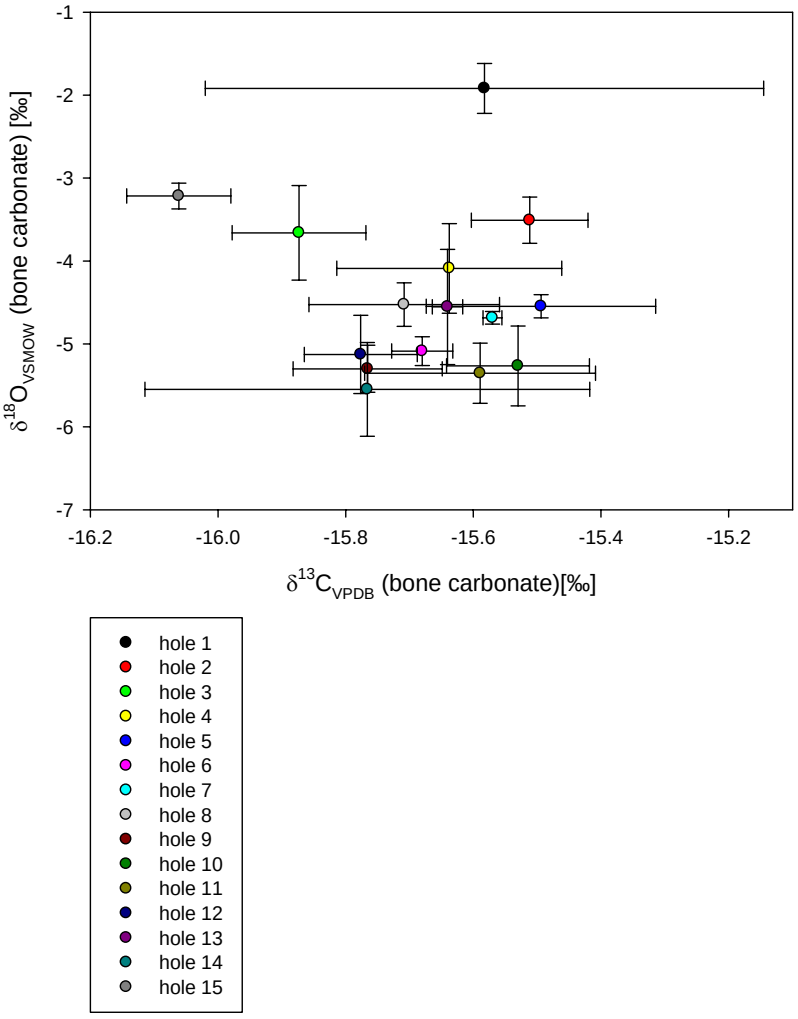

Figure 32. Graph showing the plots of mean $\delta^{13}C$ and $\delta^{18}O$ values, with error bars, for individual samples of UoD.

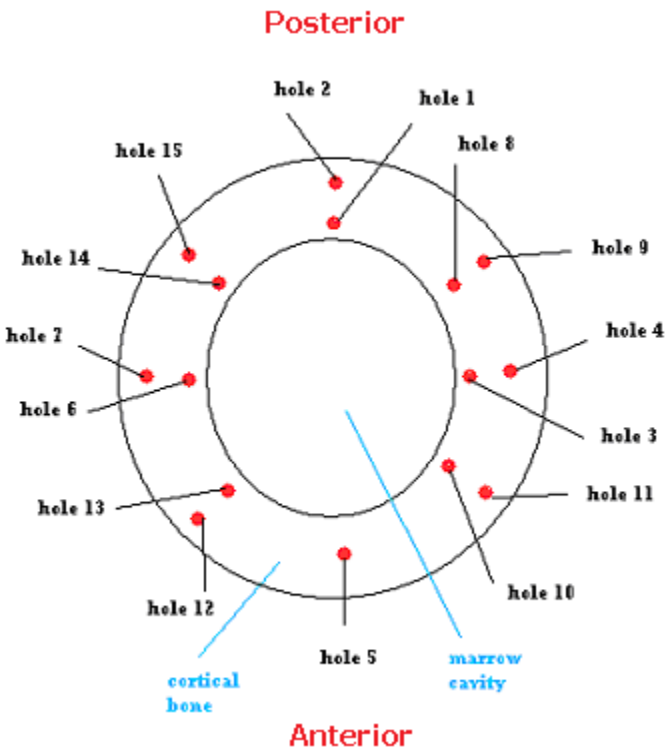

Figure 33. Schematic illustrating the locations of drilled holes from UoD.

The results from the descriptive statistical tests applied to $\delta^{13}C$ and $\delta^{18}O$ values collected from sample 820R are shown in Appendix 21. Hole number 7 had the lowest mean $\delta^{13}C$ value at -15.83‰. Hole number 6 had the highest mean $\delta^{13}C$ value (-10.6‰), with the difference between these $\delta^{13}C$ values being large, at 5.23‰. The highest mean $\delta^{18}O$ value was obtained from hole number 1 (-1.114‰), and the lowest from number 5 (-4.94‰), with a range of -3.83‰; low in comparison with the range of mean $\delta^{13}C$ values. Appendix 21 also indicates that hole number 6 has a very large standard deviation (12.86), and indicates that the data collected from this particular hole may be erroneous. The graph in Figure 34 (excluding hole 6 due to its erroneous nature) demonstrates the results of the plots of $\delta^{13}C$ and $\delta^{18}O$ mean values for each hole. It shows visually that the greatest amount of variation is in $\delta^{18}O$ values (y-axis) with the points plotted from around -1.2‰ to -5.3‰ (a range of 4.1‰), in comparison with the range for $\delta^{13}C$ values (from approximately -15‰ to -16.1‰) of 1.1‰. The closest relationship is between holes 3 and 4 for both carbon and oxygen values, and for carbon values only, holes 1 and 2. An inspection of the illustration drilled hole location on section 820R (Figure 35) shows that

these two holes are adjacent to each other, as are holes 1 and 2. One-way ANOVAs were performed to assess variation between both $\delta^{13}C$ and $\delta^{18}O$ values from all holes, the results of which can be viewed in Appendix 22. The p-value (0.549) of the $\delta^{13}C$ ANOVA suggests there is not a significant difference between the $\delta^{13}C$ values obtained from the holes drilled in 820R. A p-value of <0.001 for the ANOVA performed on $\delta^{18}O$ data indicates there is a statistically significant difference between the $\delta^{18}O$ values measured from the samples collected from 820R.

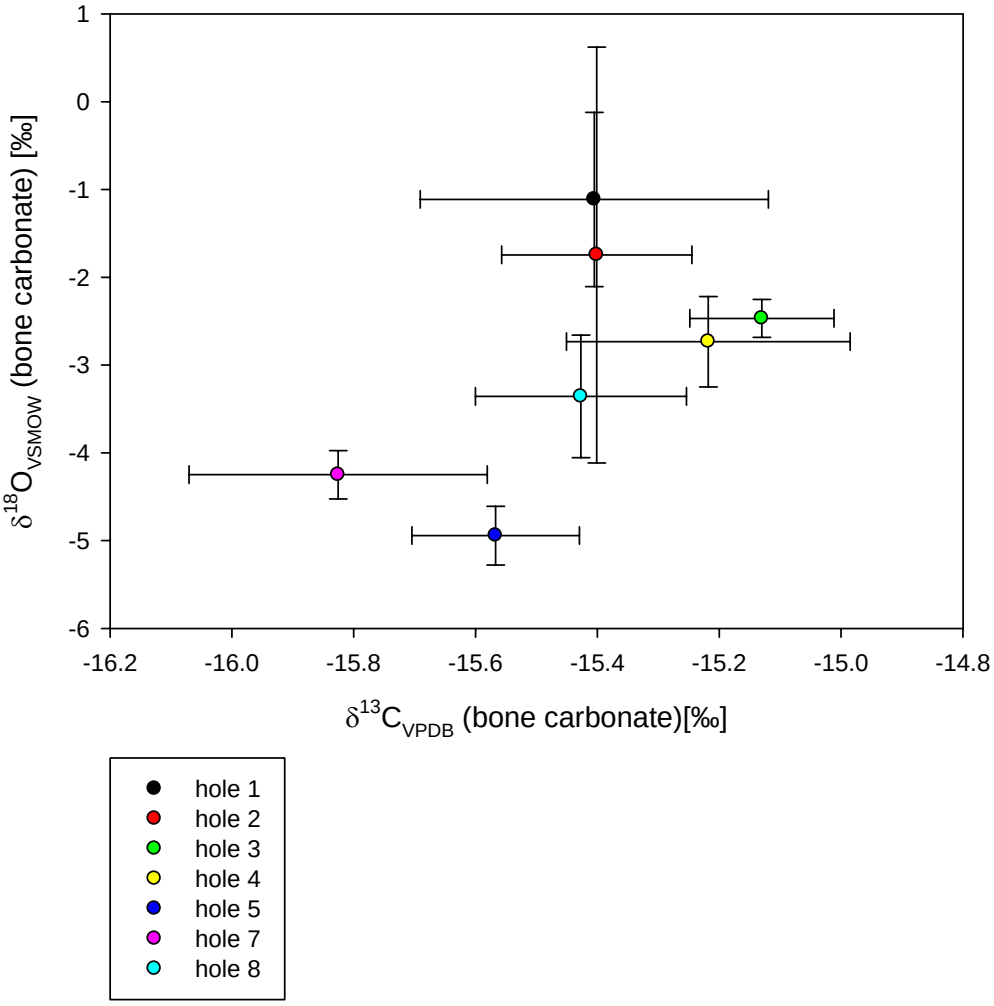

Figure 34. Graph showing the plots of $\delta^{13}C$ and $\delta^{18}O$ mean values excluding hole 6, with error bars, for individual samples of 820R.

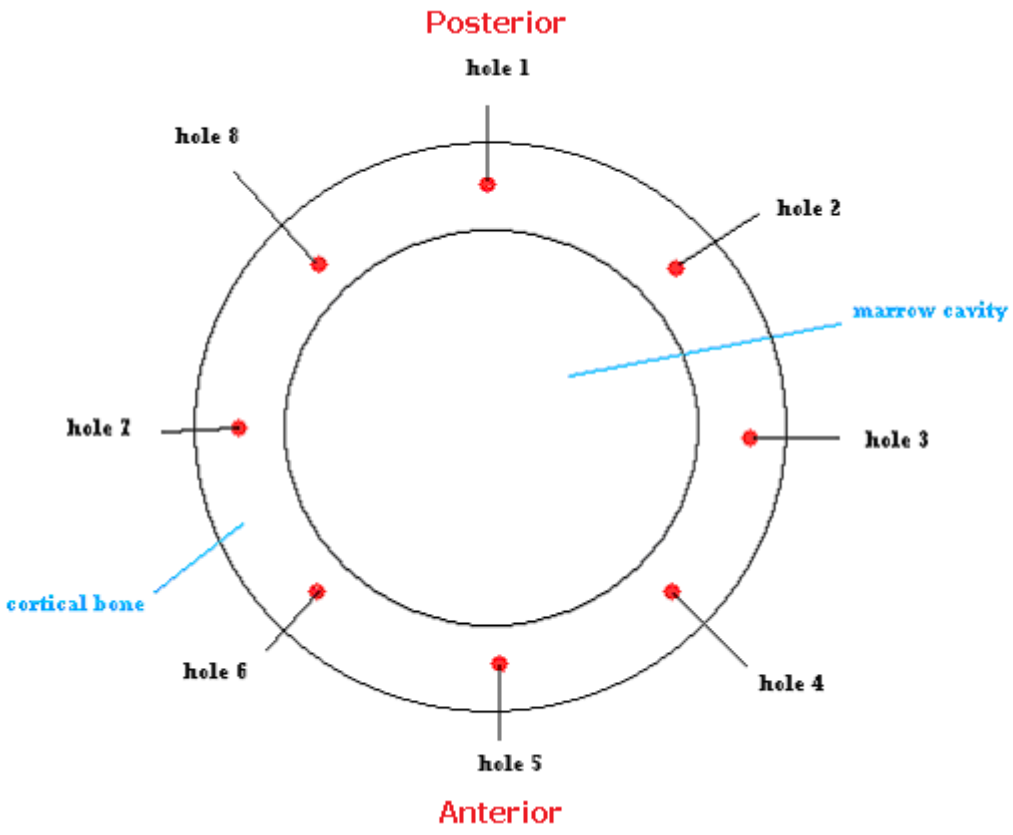

Figure 35. Schematic illustrating the locations of drilled holes from 820R

Chapter 8: Discussion

It has been demonstrated that variations in the isotopic abundance of light elements in compounds constructing human tissues (hair, nails, bones, teeth) reflect the isotopic constituents of food and drink consumed (Fraser and Meier-Augenstein, 2007; Fraser *et al.*, 2006; Nardoto *et al.*, 2006; Sharp *et al.*, 2003). Studies have also illustrated a strong relationship between the ^{18}O signature of tap water, and geographical location (Bowen *et al.*, 2007; Darling and Talbot, 2003; Ehleringer *et al.*, 2008). When an individual resides in a particular region and consumes the local tap water, the ^{18}O signature of the tap water becomes incorporated into their body tissues. Analysis of the ^{18}O content of these tissues may infer the geographical origin of an individual and subsequently assist with their identification. Research has demonstrated that despite the metabolic fractionation of oxygen occurring as it passes through the body, there is still a strong relationship between the isotopic signature of dietary water and human tissues (Longinelli, 1984). Levinson *et al*. (1987) and Luz and Kolodny (1985; 1989) have demonstrated similar strong correlations for oxygen isotopes between ingested water, and skeletal material ($R = 0.93$ for teeth ($n = 40$), and $R = 0.99$ for bone ($n = 32$)). In a similar fashion to oxygen, the isotopic signature of dietary carbon also has a strong relationship with body tissues (Harrison and Katzenberg, 2003; Lee-Thorp and Sponheimer, 2003; McCullagh *et al.*, 2005; Wright and Schwarcz, 1998). The majority of carbon is derived from the carbohydrate portion of an individual's diet, unless they are carnivorous where it originates predominantly from protein (Krueger and Sullivan, 1984). Carbohydrates are generally used for energy metabolism or converted to glycogen for storage and use at a later time. Most carbohydrates are ultimately converted to CO_2, moved around the body as blood bicarbonate (HCO_3^-), transported to the lungs, and expired through breath. This means body tissues incorporating carbon from blood bicarbonate will be influenced by the isotopic content of carbohydrates within dietary intake. One such material is hydroxyapatite which incorporates carbonate ions (HCO_3^- originating from blood bicarbonate) during crystallisation (Sullivan and Krueger, 1981); thus the isotopic

signature of dietary carbon can be calculated through stable isotope analysis of the hydroxyapatite of bone and teeth tissues.

Comparison of the mean $\delta^{13}C$ values for all bone sections (see Table 5) with the range of $\delta^{13}C$ values for C_3, CAM and C_4 plants suggested (see Chapter 4) that all individuals consumed mainly CAM (-14 to -33‰ (Bender *et al.*, 1973)) and/or C_4 type plants (-9 to -18‰ (Bender *et al.*, 1973; O'Leary, 1981)). The mean carbon values demonstrated by individuals in this study were between -12.86‰ and -16.66‰, (see Table 5). These values suggest that their diets included plants such as corn (or corn-fed beef) maize, millet and sugar cane, which are not usually associated with a C_3-based European diet (Meier-Augenstein, 2010). Other literature measuring $\delta^{13}C$ values in skeletal material support the values recorded in this study, but report a $\delta^{13}C$ enrichment of bone apatite in comparison with diet (Kosiba *et al.*, 2007). Theoretical enrichment values of +8-12‰ (Sullivan and Krueger, 1981), +11-12‰ (Hedges, 2003), and around 12‰ (Lai *et al.*, 2007) have been reported. These enrichment values can be used to calculate the $\delta^{13}C$ value of food consumed by an individual. Using the average $\delta^{13}C$ measured in this research (-14.5‰), the $\delta^{13}C$ value of dietary input ranges from -22.5 to -26.5‰. This range falls between that for C_3 plants (-22 to -34‰) reported by Bender and colleagues (1973) and O'Leary (1988), and is substantially lower than the range reported by the same authors for C_4 plants (-9 to -18‰). The results of this research are comparable with that of other studies utilising bone to measure $\delta^{13}C$ values. Kosiba and colleagues (2007) analysed the bone apatite of archaeological samples collected from 10 Viking and Early Christian individuals in Sweden. These individuals, like the cadavers sampled in this study, would have consumed a C_3 based diet. The $\delta^{13}C$ values reported range from -12. 2‰ to -14.7‰ (a difference of 2.5‰), with a mean $\delta^{13}C$ value of -13.6‰ (Kosiba *et al.*, 2007). The results collected in this research (see Table 5) fall both within and around this range (from -12.86‰ to -16.66‰, a difference of 3.8‰), the average $\delta^{13}C$ value being -14.5‰. This suggests that the cadavers sampled in this study, like those sampled by Kosiba and colleagues (2007) consumed mainly C_3 plants. This is further evidenced when these values are compared with $\delta^{13}C$ values of those with a predominantly C_4 plant diet. The average $\delta^{13}C$ values of apatite from individuals consuming mainly C_4 plants

have been reported as -9.8‰ ± 0.1 (Tykot *et al.*, 1996), -9.5 ‰ ± 1.2 (Tykot, 2002), -6.8‰ ± 1.2 (Tykot, 2002), substantially higher than the average $\delta^{13}C$ value (for probable C_3 consumers) of -14.5‰ recorded in this research.

In addition to carbon isotope values supporting data from previous studies, the results from this research also support publications suggesting a strong relationship between the $\delta^{18}O$ content of dietary water, body tissues, and geographical location. The estimated dietary water values for cadavers sampled in this study can be seen in Figure 20, and are -7.79‰ for 820R, -7.88‰ for 792L, and -6.35‰ for 792R. A total of 62 water samples collected in Dundee during this research were analysed (for comparison with the estimated $\delta^{18}O$ value of dietary water of cadavers), with the average $\delta^{18}O$ value of Dundee tap water being calculated as -7.6‰ (see Appendix 23). This average is not statistically different from that of the estimated dietary water values for the sampled cadavers, supporting previous research suggesting a link between the ^{18}O composition of dietary water and body tissues, and the equations developed to calculate dietary water values (Daux *et al.*, 2008; Iacumin *et al.*, 1996; Meier-Augenstein, 2010). The standard deviation of 1.14 may account for some of the discrepancy between the $\delta^{18}O$ values of tap water samples collected from Dundee, and the estimated $\delta^{18}O$ values of dietary water values of the cadavers.

The $\delta^{18}O$ values of both estimated dietary water and measured Dundee tap water are close to those for the corresponding areas on a map compiled by Darling *et al.* (2003) illustrating the ^{18}O composition of tap waters in the UK (see Figure 36). The values measured by Darling and colleagues (2003) however, are slightly more depleted in ^{18}O than both the estimated dietary water values, and measured values. This may be a result of differences between Dundee and the closest location sampled by Darling and colleagues (2003). The nearest site to Dundee was a spring in Drumtochty Forest providing a $\delta^{18}O$ value of -8.2‰ (Darling *et al.*, 2003). This particular location is situated inland (as opposed to Dundee located on the coastline of an estuary), is approximately 300m above sea level (the highest point in Dundee is ~150m) and around 15 miles from Dundee (see Figure 36 for approximate locations). It is known that altitude can

significantly affect the $\delta^{18}O$ values of precipitation (Aggarwal *et al.*, 2010; Dansgaard, 1964), and accordingly Darling and colleagues (2003) suggest a -0.30‰ correction for $\delta^{18}O$ values per 100m increase in altitude. When applying this calculation to the average $\delta^{18}O$ value obtained from Dundee tap water (-7.6 + (-0.3*1.5)) a $\delta^{18}O$ value of -8.1‰ is achieved; 0.1‰ more than the -8.2‰ measured at Drumtochty Forest, and indicating the results of this research support those published by Darling and colleagues (2003). The data also supports literature detailing a link between precipitation and geographical location (Bowen *et al.*, 2007; Bowen and Wilkinson, 2002).

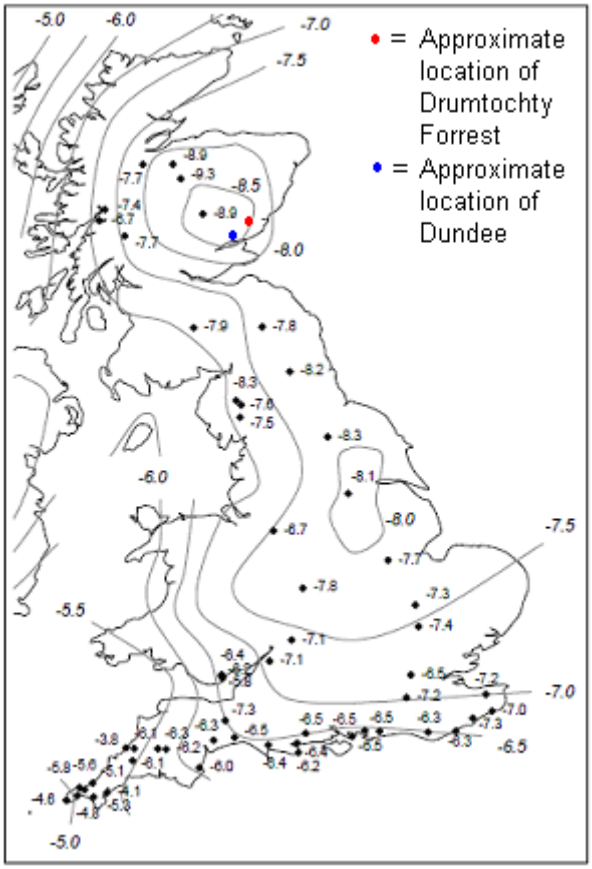

Figure 36. After Darling *et al*., (2003) $\delta^{18}O$ values of tap waters in the UK marked by diamonds. Groundwater values marked by contours.

Closer inspection of the estimated $\delta^{18}O$ value of dietary water indicates that the individuals within the study may not only have originated from the east coast of Scotland, but also some areas of Central Europe (See Figure 37). As the geographical origin of the

cadavers sampled within this study were known, they can be easily traced to the east coast of Scotland. For individuals of unknown provenance, the use of strontium isotopes in addition to oxygen isotopes could assist in estimating geographical origin. Strontium isotopes ($^{87}Sr/^{86}Sr$) have already been used extensively as a complementary source of information on geographic origins of human populations (Beard and Johnson, 2000; Burton *et al.*, 2003; Hodell *et al.*, 2004; Price *et al.*, 2004). Strontium is taken into the human body via dietary intake, and its signature dependent upon the geology (soil type, underlying rocks, weathering conditions etc) of a particular region (NERC, 2010). Plants developing in an area will have an $^{87}Sr/^{86}Sr$ ratio indicative of the soil in that location. These plants are then consumed by humans, and incorporated into the skeleton where strontium substitutes for calcium in bones and teeth. As teeth are formed during childhood, analysis of the $^{87}Sr/^{86}Sr$ content of tooth enamel can assist investigators in estimating an individual's geographical location during childhood and adolescence. As bones remodel constantly, their $^{87}Sr/^{86}Sr$ composition can be considered 'an average' signal over a lifetime (Bentley *et al.*, 2003). Since oxygen isotopes relate to hydrology and $^{87}Sr/^{86}Sr$ correlates with geology, the two isotope systems act as independent indicators for geographical locations. Regional maps of oxygen (Figure 17) and strontium isotope values (Figure 38) can be used to estimate the origin of an individual, and may be useful in determining whether the cadavers sampled in this study (if of unknown provenance) were originally from the east coast of Scotland or certain regions of Eastern Europe as demonstrated in Figure 37.

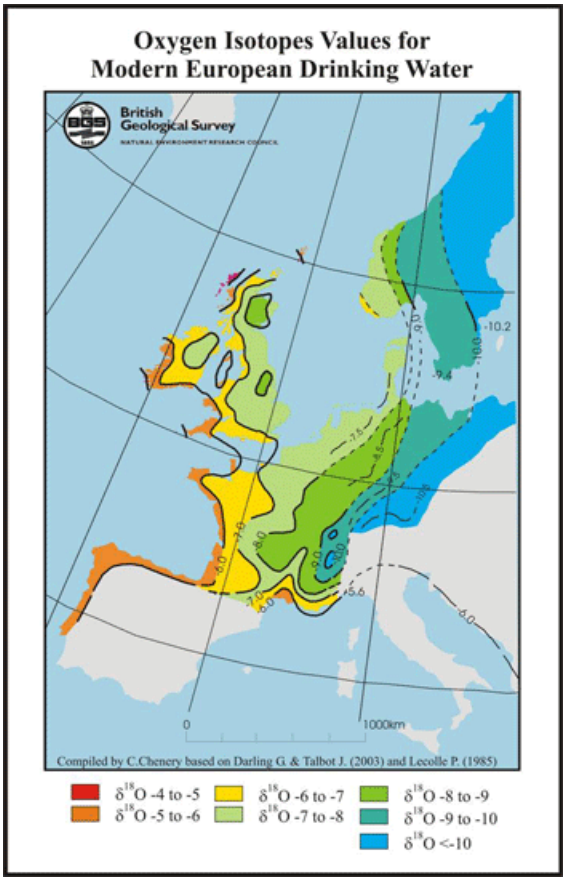

Figure 37. (NERC, 2010). Image demonstrating the $\delta^{18}O$ values of modern European drinking water.

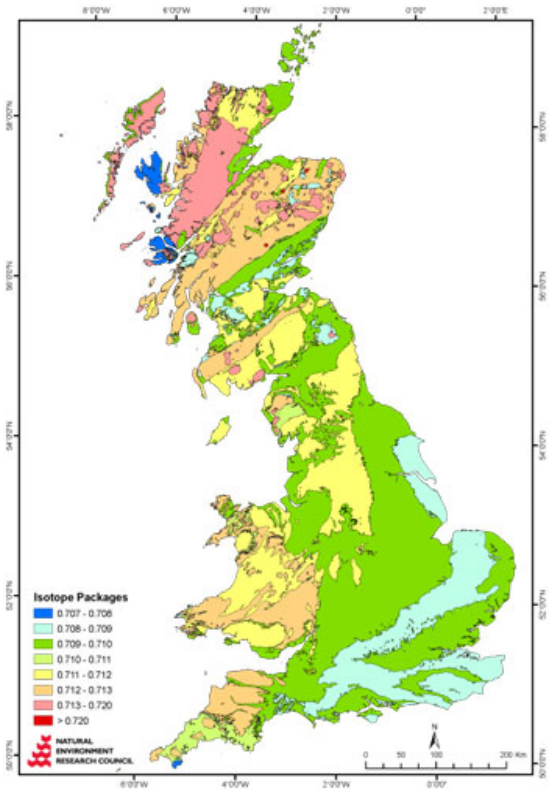

Figure 38. (NERC, 2010). Image demonstrating the spatial variations in $^{87}Sr/^{86}Sr$ in the UK.

One key aim of this research was to investigate and quantify intra- and inter-individual variation associated with human bone samples; i.e. to quantify the variability associated with bone carbonate samples collected from a single individual, and to assess whether oxygen and carbon isotopes in bone carbonate may be used to distinguish between individuals. Figure 19 suggests that $\delta^{13}C$ and $\delta^{18}O$ values are able to distinguish between individuals; however statistical analysis demonstrates that inter-individual variability in these isotopes may not be enough to distinguish between persons (Tables 6 and 7). An investigation of all data indicates that the most variable isotope in bone carbonate is ^{18}O (Tables 6 and 7), which has greater inter-individual variability than ^{13}C, and suggests it would be most useful (of the stable isotopes of these two elements) for distinguishing between individuals. All samples with the exception of KAS (and RPT) show the majority of variation in oxygen isotope measurements, as opposed to carbon isotope measurements. Other studies measuring $\delta^{13}C$ and $\delta^{18}O$ values in skeletal tissues also report the majority of variation to occur in oxygen isotopes (Bentley and Knipper, 2005;

Lai *et al.*, 2007; Lee-Thorp and Sponheimer, 2003). This may be due to the fact that the sole source of carbon forming human tissue originates from the diet, and the possible variation in carbon δ values is from around -9‰ to approximately -34‰ (the range of values for C_3, C_4 and CAM plants ~25‰). The range of $\delta^{18}O$ values is far greater; for example from ocean water (0‰) to water at the poles (-50‰). The number of ^{18}O sources forming body tissues is also greater; the sole source of carbon originates from solid food intake, whereas there are a number of oxygen sources contributing to body tissues including inspiration, water, and oxygen within solid foods. The greater variation displayed by oxygen isotopes may also be a result of more rapid turnover of water than solid food (Astrup and Tremblay, 2009; Shimamoto and Komiya, 2000), leading to more dynamic changes in dietary oxygen than carbon isotopes.

Regression analysis of $\delta^{13}C$ and $\delta^{18}O$ values ($r^2 = 0.194$) indicated that there is no correlation between the $\delta^{13}C$ and $\delta^{18}O$ values within skeletal carbonate, which is contradictory to some studies. Lai and colleagues (2007) studied ancient Sardinian skeletal remains (n = 75, dated 2500-1300 BC) and found a 'strong linear correlation' (r value not published) between the ^{18}O and ^{13}C content of bone carbonate. It is acknowledged by the authors that the individuals sampled would not have migrated great distances, nor substantially altered their dietary intake. This may be the reason for the lack of correlation between $\delta^{18}O$ and $\delta^{13}C$ values measured in this research. The individuals sampled in this study would have had the opportunity to travel across the country and even had access to global travel. Movement between different geographical areas may have contributed to the lack of correlation between the two isotopes. It is however important to note that the average $\delta^{18}O$ values (indicative of migration) between cadaver 792 and 820 do not demonstrate a significant difference (see Table 7), and it is therefore unlikely that travel would have contributed to the lack of correlation. Other studies utilising tooth enamel carbonate (as opposed to bone carbonate) have reported little correlation between ^{13}C and ^{18}O isotopes. Wright and Schwarcz (1998) analysed the carbon and oxygen isotope content of 104 teeth from 41 Guatemalan prehistoric skeletons and found a plot of the $\delta^{18}O$ and $\delta^{13}C$ values from all samples to demonstrate a 'broad variation' (r value not published). In a similar fashion, a study of Neolithic pig enamel (n

= 44) by Bentley and Knipper (2005) also demonstrated a poor correlation between $\delta^{13}C$ and $\delta^{18}O$ values (r value not published).

To assess intra-individual variability several holes were drilled in each femoral section and the content of each one analysed for its $\delta^{13}C$ and $\delta^{18}O$ value. Some of the holes within each bone section (those of X65 D10, JR_3 14, and KAS2 and RPT) demonstrated a statistically significant difference between $\delta^{13}C$ and $\delta^{18}O$ values (for example see Appendix 10). Other bone sections demonstrated a variation in $\delta^{18}O$ values only (792R, UoD and 820R), and 792L had statistically significant differences in $\delta^{13}C$ values. The main variation within the isotopes of bone carbonate (when considering all bone sections) is that of the ^{18}O composition, again this is likely to be due to the possible number of sources and turnover rate of oxygen isotopes. In addition to these results, it was also found that there is no link between the isotopic content of a sample and the location on the bone the sample was collected from, or which leg of an individual was sampled. This is to be expected due to the nature of bone growth and remodelling. Bone (as mentioned in Chapter 5) develops through the production of osseous tissue by osteoblasts located within osteons, which eventually become inactive osteocytes (see Figure 10) (Carter, 1984; Hill, 1998). Figure 10 illustrates the random nature of both the size and shape of osteons throughout cortical bone. When drilling the holes required to sample the carbonate, they would have been drilled across a varying number of osteons on each occasion. Each of these osteons may have a slightly different isotopic value resulting from the varying times the osteoblasts within them were active (and thus producing new bone tissue) (Hill, 1998). For example, increased stress on one side of the femur will in turn, increase the amount of osteoblast activity (and thus bone production) in the area of stress (Carter, 1984; Mundy, 1994). The new bone material (located on one side of the femur) may have an isotopic content indicative of the signature of water and food consumed at the time of stress, whereas the rest of the bone material may display isotopic values suggestive of dietary intake over the previous 10 years. This may be the reason for significant variation in the isotopic content of samples collected from different locations on the same section of femur. It is therefore important to consider the isotopic values obtained from skeletal material to be an average from throughout an individual's life.

Unfortunately the collection of several samples from various locations on a section of bone has never been undertaken before, and so cannot be compared with other data. It is suggested that as there is no link between the location of sampling and the measured $\delta^{13}C$ or $\delta^{18}O$ isotope value. This was evidenced by the variation of samples collected from different holes drilled in the same femoral section. It is suggested that future samples be collected in the region between the marrow cavity and the linea aspera (see Figure 18). This is where the distance between the inner (lining of the marrow cavity) and outer cortex (outer most lining of the cortical bone) is at its greatest, meaning the possibility of contamination of inorganic carbonate from organic components is minimal.

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Chapter 9: Conclusion

In recent times, headlines have been dominated by mass disaster incidents such as the Asian tsunami (2004) and the London tube and bus bombings (2005). The field of human identification requires the development of new methods able to overcome problems associated with traditional techniques such as degradation of DNA and fragmentation. One such method, with the potential to establish the geographical origin or recent movements of an individual, is stable isotope profiling. This technique has the ability to utilize the relationships between isotopic content of an individual's diet, the isotopic composition of their body tissues (such as hair and bones), and geo-location or recent travels. Simply expressed, this technique has the potential to map an individual's past through isotopic analysis of their body tissues – otherwise known as 'human provenancing'. It is a rapid, cost effective, and accurate, and may be of assistance to forensic investigators in identification of living and deceased individuals.

Variation in isotopic compositions arises from a process known as fractionation. Evaporative and condensative processes during the hydrologic cycle alter the isotopic signature of water by favouring the light and heavy isotopes respectively. Evaporation and condensation occur constantly as water is transported across the globe, and result in tap water from different geographical areas varying in isotopic content. This water is then consumed as part of dietary intake, and incorporated into the human body during tissue formation. However, before the isotopic composition of water is built into human material, it undergoes further fractionation as a result of metabolic processes within the human body. The rate of metabolism can vary substantially both between individuals and within the tissues of the same individual. This is an important consideration when applying stable isotope profiling to body tissues in forensic investigations, as it is likely there will be inter- and intra-individual variability for isotopic compositions.

Unfortunately, little data is available regarding inter- and intra subject variability in SIPs. This is a current limitation, as information would be used to determine the probative

value of the evidence produced. For example, judges evaluate the errors and variability of a method before determining whether to declare complex scientific evidence admissible in Court, and barristers use the figures to either support or undermine the credibility of evidence proffered. It is therefore crucial that this area of profiling is explored further and reliable, quantifiable results are produced that can be of probative value in the judicial system.

The primary aim of this research was to quantify the inter- and intra-individual variation associated with human tissue, in particular skeletal material. This was achieved by collecting femoral sections from cadavers and analysing the bone carbonate for its $\delta^{13}C$ and $\delta^{18}O$ values. Intra-individual variability of skeletal material was assessed by collecting a number of samples from the same femoral section and comparing the means using an ANOVA. Examination of the ^{13}C and ^{18}O content from sections sampled from the left and right legs of the same cadaver also assisted in the assessment of the variation within an individual. Inter-individual variability was investigated by a comparison of the mean $\delta^{13}C$ and $\delta^{18}O$ values with the same information obtained from bone sections originating from a number of cadavers. The $\delta^{18}O$ values from individuals were compared with established precipitation maps with a view to examining the relationship between the ^{18}O content of bone carbonate, and local precipitation. The ^{13}C content was used to determine what photosynthetic pathway the majority of plants consumed by the individual had undertaken (i.e. C_3, C_4 or CAM).

The data demonstrated that the vast majority of variation occurs in oxygen isotopes as opposed to carbon isotopes, which is supported by other literature. This variation may be a result of the greater number of oxygen sources contributing to the isotopic signature of human tissue, or the more rapid turnover of water (and thus oxygen isotopes) in the body. The results also suggested there is very little variation in terms of the isotopic composition between left and right femora of a single individual, but a significant difference (the majority of which in oxygen isotopes) when sampling a small piece of a single femur in several different locations. This was evident when analysing the material taken from holes drilled in several locations on a single femoral section. It was noted that

there is no relationship between the position of the sampling site and variation in isotopic content. These variations may be a result of the irregular growth pattern of human bone. Correlation between ^{13}C and ^{18}O isotopes ($r^2 = 0.194$) was poor, with these results being corroborated by some studies but undermined by others. Dietary intake of the individuals sampled in this research indicated that they consumed a predominantly C_3 plant-based diet, a result supported by other literature investigating relationships between $\delta^{13}C$ values of bone carbonate and dietary intake of Europeans. In addition, the data collected suggested a link between the ^{18}O composition of dietary water, body tissues and geographical location; a relationship that has been studied (and corroborated) by other authors.

It is acknowledged that the small number of samples utilised in this research may result in misleading conclusions from the data. In particular, the number of bone and hair samples analysed requires increasing to investigate the true inter- and intra-individual variability associated with these tissues. It is also understood that the samples in this research have originated from individuals residing in a very small geographical area. This study however has provided preliminary information with regard to differences in isotopic composition, and with a greater number of samples analysed, will hopefully permit the deduction of more accurate and meaningful conclusions. It is therefore recommended that the next stage of this research dedicate itself to the collection and analysis of bone material from individuals originating from a variety of geographical locations, and the analysis of those bone samples already collected.

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Appendices

Appendix 1: Table describing the cadavers sampled for femoral sections

Number	Sex	Age	Cause of Death (COD)	Most Recent Residence
820	1	95	chronic obstructive pulmonary disease	Kirkcaldy
792	0	57	end stage renal disease	Dundee

Appendix 2: Tables presenting the raw data collected from $\delta^{13}C$ and ^{18}O isotope analysis of the all bone sections

Sample 792L

Sample Name	Mass (g)	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS_19		2.851	1.093		
NBS_19-1		3.038	0.743	2.906	0.599
NBS_19-2		2.830	-0.038	0.155	0.579
NBS_19		2.660	0.762		
NBS_19-1		2.607	-0.069	2.676	0.123
NBS_19-2		2.760	-0.324	0.078	0.568
792 L_1A	10.39	-12.755	-1.233		
792 L_1A-1	10.39	-12.386	-1.646	-12.584	-1.458
792 L_1A-2	10.39	-12.612	-1.495	0.186	0.209
792 L_1B	11.73	-12.085	-3.649		
792 L_1B-1	11.73	-12.084	-3.806	-12.138	-3.781
792 L_1B-2	11.73	-12.244	-3.889	0.092	0.122
792 L_2A	7.01	-12.928	-2.887		
792 L_2A-1	7.01	-12.880	-2.172	-12.887	-2.486
792 L_2A-2	7.01	-12.854	-2.400	0.038	0.365
792 L_2B	5.96	-12.743	-2.474		
792 L_2B-1	5.96	-12.604	-2.627	-12.778	-2.712
792 L_2B-2	5.96	-12.988	-3.034	0.194	0.289
792 L_3A	6.70	-13.341	-3.012		
792 L_3A-1	6.70	-13.250	-3.651	-13.440	-3.405
792 L_3A-2	6.70	-13.730	-3.551	0.255	0.344
792 L_3B	9.48	-13.519	-3.229		
792 L_3B-1	9.48	-13.194	-3.037	-13.355	-3.088
792 L_3B-2	9.48	-13.353	-2.997	0.163	0.124
792 L_4A	7.23	-13.582	-2.259		
792 L_4A-1	7.23	-13.707	-1.584	-13.553	-1.956
792 L_4A-2	7.23	-13.370	-2.026	0.17	0.343
792 L_4B	4.15	-13.456	-3.296		
792 L_4B-1	4.15	-13.517	-2.348	-13.435	-2.785
792 L_4B-2	4.15	-13.331	-2.712	0.095	0.478
Bicarb_X		-3.270	-11.849		

Bicarb_X-1		-3.310	-12.321	-3.234	-12.102
Bicarb_X-2		-3.123	-12.137	0.098	0.238
Bicarb_X		-3.332	-12.048		
Bicarb_X-1		-3.372	-11.940	-3.332	-12.101
Bicarb_X-2		-3.293	-12.315	0.040	0.193
792 L_5A	7.10	-12.809	-3.119		
792 L_5A-1	7.10	-12.815	-1.085	-12.896	-2.426
792 L_5A-2	7.10	-13.065	-3.074	0.146	1.162
792 L_5B	8.74	-12.087	-4.222		
792 L_5B-1	8.74	-11.989	-3.643	-12.034	-4.06
792 L_5B-2	8.74	-12.027	-4.315	0.049	0.364
792 L_6A	8.1	-13.522	-3.730		
792 L_6A-1	8.1	-13.393	-3.932	-13.387	-3.738
792 L_6A-2	8.1	-13.246	-3.552	0.138	0.19
792 L_6B	7.83	-13.613	-3.121		
792 L_6B-1	7.83	-13.582	-2.574	-13.593	-2.731
792 L_6B-2	7.83	-13.583	-2.498	0.018	0.340
792 L_7A	9.39	-12.928	-3.818		
792 L_7A-1	9.39	-12.911	-3.681	-12.967	-3.744
792 L_7A-2	9.39	-13.061	-3.734	0.082	0.069
792 L_7B	10.56	-12.612	-2.164		
792 L_7B-1	10.56	-12.761	-2.004	-12.672	-1.631
792 L_7B-2	10.56	-12.643	-0.725	0.079	0.789
792 L_8A	8.35	-12.601	-4.034		
792 L_8A-1	8.35	-12.517	-3.772	-12.589	-3.955
792 L_8A-2	8.35	-12.650	-4.058	0.067	0.159
792 L_8B	10.21	-12.463	-3.907		
792 L_8B-1	10.21	-12.306	-3.704	-12.362	-3.669
792 L_8B-2	10.21	-12.317	-3.397	0.088	0.257
LSVEC		-44.549	-26.969		
LSVEC-1		-44.680	-26.851	-44.627	-27.024
LSVEC-2		-44.651	-27.252	0.069	0.206
LSVEC		-44.669	-27.039		
LSVEC-1		-44.669	-27.268	-44.694	-27.042
LSVEC-2		-44.743	-26.819	0.043	0.225

Sample 792R

Sample Name	Mass (g)	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS_19		2.711	0.512		
NBS_19-1		2.884	-0.294	2.827	-0.126
NBS_19-2		2.887	-0.596	0.101	0.573
NBS_19		2.646	-0.779		
NBS_19-1		2.829	-0.952	2.753	-0.941
NBS_19-2		2.784	-1.092	0.095	0.157
792R_1A	5.90	-12.504	-2.110		
792R_1A-1	5.90	-13.046	-2.839	-12.738	-2.557
792R_1A-2	5.90	-12.664	-2.721	0.278	0.391

792R_1B	6.41	-13.118	-1.011		
792R_1B-1	6.41	-12.617	-0.654	-12.848	-0.719
792R_1B-2	6.41	-12.808	-0.493	0.253	0.265
792R_2A	5.02	-12.391	-2.859		
792R_2A-1	5.02	-11.789	-3.910	-12.175	-3.321
792R_2A-2	5.02	-12.345	-3.193	0.335	0.537
792R_2B	4.33	-13.243	-2.346		
792R_2B-1	4.33	-13.604	-2.084	-13.445	-2.312
792R_2B-2	4.33	-13.488	-2.506	0.184	0.213
792R_3A	5.16	-12.791	-2.816		
792R_3A-1	5.16	-12.832	-2.859	-12.734	-2.805
792R_3A-2	5.16	-12.579	-2.740	0.136	0.060
792R_3B	6.47	-13.103	-2.233		
792R_3B-1	6.47	-12.681	-1.994	-12.847	-2.121
792R_3B-2	6.47	-12.756	-2.135	0.225	0.120
792R_4A	5.98	-13.441	-3.007		
792R_4A-1	5.98	-13.076	-2.574	-13.276	-2.662
792R_4A-2	5.98	-13.310	-2.285	0.185	0.363
792R_4B	6.84	-12.842	-3.399		
792R_4B-1	6.84	-12.710	-1.274	-12.768	-2.654
792R_4B-2	6.84	-12.752	-3.289	0.067	1.196
Bicarb_X		-3.277	-12.351		
Bicarb_X-1		-3.192	-12.444	-3.285	-12.361
Bicarb_X-2		-3.386	-12.288	0.097	0.078
Bicarb_X		-3.304	-12.912		
Bicarb_X-1		-3.251	-12.631	-3.302	-12.689
Bicarb_X-2		-3.350	-12.524	0.05	0.200
792R_5A	6.96	-12.854	-1.783		
792R_5A-1	6.96	-12.917	-2.361	-12.515	-2.186
792R_5A-2	6.96	-11.773	-2.415	0.643	0.350
792R_5B	7.72	-13.204	-3.009		
792R_5B-1	7.72	-12.989	-3.420	-13.273	-3.149
792R_5B-2	7.72	-13.627	-3.018	0.325	0.235
792R_6A	6.02	-12.682	-2.555		
792R_6A-1	6.02	-12.710	-2.978	-12.677	-2.783
792R_6A-2	6.02	-12.638	-2.816	0.036	0.213
792R_6B	6.50	-12.836	-2.573		
792R_6B-1	6.50	-12.793	-2.877	-12.944	-2.512
792R_6B-2	6.50	-13.204	-2.113	0.226	0.385
792R_7A	6.98	-12.765	-3.208		
792R_7A-1	6.98	-12.784	-3.485	-12.769	-3.361
792R_7A-2	6.98	-12.759	-3.391	0.013	0.141
792R_7B	6.52	-12.406	-3.036		
792R_7B-1	6.52	-12.619	-3.837	-12.621	-3.71
792R_7B-2	6.52	-12.839	-4.258	0.217	0.621
792R_8A	7.10	-13.021	-3.930		
792R_8A-1	7.10	-13.121	-4.165	-13.078	-4.016
792R_8A-2	7.10	-13.093	-3.954	0.052	0.129

792R_8B	7.22	-13.144	-4.234		
792R_8B-1	7.22	-13.109	-4.324	-13.114	-4.359
792R_8B-2	7.22	-13.089	-4.519	0.028	0.146
LSVEC		-44.690	-26.984		
LSVEC-1		-44.757	-27.013	-44.723	-27.001
LSVEC-2		-44.721	-27.007	0.034	0.015
LSVEC		-44.716	-27.153		
LSVEC-1		-44.475	-27.059	-44.571	-27.069
LSVEC-2		-44.523	-26.994	0.128	0.08

Sample 820R

Name	Mass (g)	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS_19		2.936	0.990		
NBS_19-1		2.801	0.248	2.868	0.434
NBS_19-2		2.867	0.064	0.068	0.490
NBS_19		2.962	0.459		
NBS_19-1		3.521	-0.217	3.089	-0.100
NBS_19-2		2.785	-0.542	0.384	0.511
820R_1A	6.55	-15.791	0.062		
820R_1A-1	6.55	-15.727	-0.217	-15.540	-0.384
820R_1A-2	6.55	-15.102	-0.542	0.381	0.391
820R_1B	7.27	-15.327	-2.224		
820R_1B-1	7.27	-15.195	-1.882	-15.270	-1.996
820R_1B-2	7.27	-15.289	-1.881	0.068	0.198
820R_2A	6.52	-15.547	-2.044		
820R_2A-1	6.52	-15.416	-2.505	-15.483	-2.643
820R_2A-2	6.52	-15.485	-3.381	0.066	0.679
820R_2B	5.44	-15.364	-2.628		
820R_2B-1	5.44	-15.110	-2.919	-15.326	-2.849
820R_2B-2	5.44	-15.485	3.000	0.199	0.196
820R_3A	6.57	-15.090	-2.392		
820R_3A-1	6.57	-15.336	-2.209	-15.179	-2.434
820R_3A-2	6.57	-15.110	-2.700	0.137	0.248
820R_3B	7.04	-15.051	-2.638		
820R_3B-1	7.04	-15.004	-2.627	-15.082	-2.503
820R_3B-2	7.04	-15.190	-2.243	0.097	0.225
820R_4A	6.00	-15.011	-3.419		
820R_4A-1	6.00	-15.105	-2.767	-15.067	-3.100
820R_4A-2	6.00	-15.236	-3.114	0.191	0.326
820R_4B	6.99	-15.011	-1.944		
820R_4B-1	6.99	-15.327	-2.443	-15.319	-2.368
820R_4B-2	6.99	-15.618	-2.716	0.304	0.391
Bicarb_X		-3.419	-12.032		
Bicarb_X-1		-3.303	-12.334	-3.328	-12.177
Bicarb_X-2		-3.261	-12.165	0.082	0.151
Bicarb_X		-3.482	-12.502		
Bicarb_X-1		-3.240	-12.376	-3.316	-12.547

Bicarb_X-2		-3.227	-12.764	0.144	0.198
820R_5A	6.72	-15.384	-4.892		
820R_5A-1	6.72	-15.437	-5.270	-15.498	-4.877
820R_5A-2	6.72	-15.674	-4.468	0.154	0.401
820R_5B	6.82	-15.669	-5.228		
820R_5B-1	6.82	-15.528	-5.165	-15.636	-5.009
820R_5B-2	6.82	-15.711	-4.634	0.096	0.326
820R_6A	4.65	-15.770	-2.216		
820R_6A-1	4.65	-15.562	-2.767	-15.654	-2.574
820R_6A-2	4.65	-15.631	-2.739	0.106	0.31
820R_6B	5.35	-16.190	-2.727		
820R_6B-1	5.35	15.654	-3.060	-15.982	-2.96
820R_6B-2	5.35	-16.102	-3.093	0.287	0.202
820R_7A	5.50	-15.900	-4.319		
820R_7A-1	5.50	-15.850	-4.739	-15.982	-4.379
820R_7A-2	5.50	-16.197	-4.079	0.188	0.334
820R_7B	6.45	-15.891	-4.126		
820R_7B-1	6.45	-15.622	-3.953	-15.669	-4.12
820R_7B-2	6.45	-15.494	-4.281	-0.203	0.164
820R_8A	6.42	-15.218	-4.128		
820R_8A-1	6.42	-15.508	-4.095	-15.346	-3.964
820R_8A-2	6.42	-15.312	-3.668	0.148	0.257
820R_8B	7.48	-15.561	-2.518		
820R_8B-1	7.48	-15.305	-2.953	-15.508	-2.75
820R_8B-2	7.48	-15.659	-2.779	0.183	0.219
LSVEC		-44.580	-26.888		
LSVEC-1		-44.717	-27.184	-44.632	-26.997
LSVEC-2		-44.600	-26.918	0.074	0.613
LSVEC		-44.669	-26.726		
LSVEC-1		-44.691	-27.102	-44.67	-26.988
LSVEC-2		-44.649	-27.137	0.021	0.228

Sample KAS2

Sample Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS19	0.640	-1.561		
NBS19-1	0.543	-2.156	0.518	-1.865
NBS19-2	0.372	-1.877	0.136	0.298
NBS19	0.572	-2.329		
NBS19-1	0.273	-2.005	0.468	-2.018
NBS19-2	0.558	-1.720	0.169	0.305
KAS2_1a	-13.820	-4.160		
KAS2_1a-1	-13.583	-3.755	-13.779	-3.991
KAS2_1a-2	-13.934	-4.059	0.179	0.211
KAS2_1b	-16.076	-4.525		
KAS2_1b-1	-15.727	-3.045	-15.670	-3.632
KAS2_1b-2	-15.208	-3.325	0.437	0.786
KAS2_2a	-15.860	-6.052		

KAS2_2a-1	-15.553	-8.131	-15.944	-6.496
KAS2_2a-2	-16.418	-5.304	0.439	1.465
KAS2_2b	-15.003	-7.426		
KAS2_2b-1	-15.470	-8.539	-15.311	-7.899
KAS2_2b-2	-15.461	-7.733	0.267	0.575
KAS2_3a	-17.325	-5.881		
KAS2_3a-1	-16.885	-6.402	-17.249	-6.420
KAS2_3a-2	-17.536	-6.976	0.332	0.548
KAS2_3b	-17.056	-5.472		
KAS2_3b-1	-17.875	-7.182	-17.299	-5.937
KAS2_3b-2	-16.965	-5.157	0.501	1.090
KAS2_4a	-17.582	-6.389		
KAS2_4a-1	-17.614	-6.602	-17.613	-6.661
KAS2_4a-2	-17.643	-6.991	0.031	0.305
KAS2_4b	-17.002	-6.791		
KAS2_4b-1	-17.570	-7.578	-17.074	-7.150
KAS2_4b-2	-16.649	-7.082	0.465	0.398
bicarb-x	-5.621	-12.453		
bicarb-x-1	-5.984	-12.865	-5.794	-12.788
bicarb-x-2	-5.776	-13.045	0.182	0.303
bicarb-x	-5.383	-12.818		
bicarb-x-1	-5.811	-13.071	-5.604	-12.988
bicarb-x-2	-5.617	-13.076	0.214	0.148
KAS2_6a	-16.848	-7.170		
KAS2_6a-1	-17.020	-8.549	-16.915	-7.444
KAS2_6a-2	-16.876	-6.612	0.092	0.997
KAS2_6b	-17.086	-7.575		
KAS2_6b-1	-17.266	-6.816	-17.243	-7.444
KAS2_6b-2	-17.376	-7.942	0.146	0.574
KAS2_7a	-16.033	-7.020		
KAS2_7a-1	-15.909	-6.983	-16.014	-7.194
KAS2_7a-2	-16.100	-7.580	0.097	0.335
KAS2_7b	-16.255	-6.586		
KAS2_7b-1	-16.509	-6.811	-16.524	-6.557
KAS2_7b-2	-16.807	-6.273	0.276	0.270
KAS2_8a	-16.205	-6.322		
KAS2_8a-1	-16.593	-6.722	-16.370	-6.405
KAS2_8a-2	-16.313	-6.170	0.200	0.285
KAS2_8b	-15.678	-6.936		
KAS2_8b-1	-15.519	-8.586	-15.590	-7.461
KAS2_8b-2	-15.572	-6.861	0.081	0.975
KAS2_9a	-16.370	-6.507		
KAS2_9a-1	-17.329	-6.515	-17.111	-6.550
KAS2_9a-2	-17.635	-6.627	0.660	0.067
KAS2_9b	-15.744	-7.070		
KAS2_9b-1	-15.957	-8.271	-15.790	-7.682
KAS2_9b-2	-15.668	-7.706	0.150	0.601
LSVEC	-46.987	-27.542		

LSVEC-1	-46.679	-27.751	-46.889	-27.777
LSVEC-2	-47.000	-28.039	0.182	0.250
LSVEC	-46.330	-27.623		
LSVEC-1	-46.535	-28.071	-46.458	-27.743
LSVEC-2	-46.508	-27.534	0.111	0.288

Sample KAS2 RPT

Sample Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS19	0.752	-1.054		
NBS19-1	0.513	-0.890	0.621	-1.133
NBS19-2	0.598	-1.456	0.121	0.291
NBS19	0.069	-2.817		
NBS19-1	0.279	-3.124	0.172	-2.936
NBS19-2	0.169	-2.866	0.105	0.165
KAS2 RPT_1a	-16.613	-4.750		
KAS2 RPT _1a-1	-16.871	-4.713	-16.655	-4.889
KAS2 RPT _1a-2	-16.480	-5.203	0.199	0.273
KAS2 RPT _1b	-17.082	-5.885		
KAS2 RPT _1b-1	-16.980	-6.180	-16.984	-6.208
KAS2 RPT _1b-2	-16.891	-6.560	0.096	0.338
KAS2 RPT _2a	-17.590	-5.860		
KAS2 RPT _2a-1	-17.293	-5.372	-17.389	-5.929
KAS2 RPT _2a-2	-17.285	-6.554	0.174	0.594
KAS2 RPT _2b	-17.098	-5.899		
KAS2 RPT _2b-1	-17.287	-7.266	-17.272	-6.647
KAS2 RPT _2b-2	-17.431	-6.776	0.167	0.693
KAS2 RPT _3a	-17.253	-7.207		
KAS2 RPT _3a-1	-17.248	-7.206	-17.288	-7.050
KAS2 RPT _3a-2	-17.364	-6.738	0.066	0.270
KAS2 RPT _3b	-16.902	-6.647		
KAS2 RPT _3b-1	-17.080	-6.370	-17.027	-6.824
KAS2 RPT _3b-2	-17.098	-7.456	0.108	0.564
KAS2 RPT _4a	-16.261	-7.437		
KAS2 RPT _4a-1	-16.940	-7.436	-16.735	-7.484
KAS2 RPT _4a-2	-17.004	-7.579	0.412	0.082
KAS2 RPT _4b	-17.471	-6.373		
KAS2 RPT _4b-1	-16.854	-5.781	-17.398	-6.569
KAS2 RPT _4b-2	-17.868	-7.552	0.511	0.902
bicarb-x	-5.917	-12.702		
bicarb-x-1	-5.882	-12.894	-5.909	-12.924
bicarb-x-2	-5.928	-13.176	0.024	0.238
bicarb-x	-5.606	-12.604		
bicarb-x-1	-5.746	-13.005	-5.724	-13.012
bicarb-x-2	-5.820	-13.426	0.109	0.411
KAS2 RPT _6a	-15.807	-7.655		
KAS2 RPT _6a-1	-16.415	-6.653	-15.779	-7.086
KAS2 RPT _6a-2	-15.116	-6.950	0.650	0.515
KAS2 RPT _6b	-17.455	-7.672		
KAS2 RPT _6b-1	-16.779	-7.330	-17.275	-7.297

KAS2 RPT _6b-2	-17.590	-6.888	0.435	0.393
KAS2 RPT _7a	-16.583	-7.178		
KAS2 RPT _7a-1	-16.761	-7.409	-16.640	-7.386
KAS2 RPT _7a_2	-16.575	-7.572	0.105	0.198
KAS2 RPT _7b	-16.379	-7.993		
KAS2 RPT _7b-1	-16.657	-8.433	-16.514	-8.074
KAS2 RPT _7b-2	-16.507	-7.796	0.139	0.326
KAS2 RPT _8a	-17.219	-7.026		
KAS2 RPT _8a-1	-17.148	-6.975	-17.180	-7.151
KAS2 RPT _8a-2	-17.173	-7.452	0.036	0.262
KAS2 RPT _8b	-17.036	-5.786		
KAS2 RPT _8b-1	-17.261	-7.937	-17.127	-6.909
KAS2 RPT _8b-2	-17.084	-7.005	0.119	1.079
KAS2 RPT _9a	-17.262	-8.092		
KAS2 RPT _9a-1	-17.324	-7.531	-17.297	-7.700
KAS2 RPT _9a-2	-17.304	-7.477	0.032	0.341
KAS2 RPT _9b	-17.224	-6.611		
KAS2 RPT _9b-1	-17.132	-6.733	-17.166	-6.742
KAS2 RPT _9b-2	-17.143	-6.882	0.050	0.136
LSVEC	-45.748	-27.215		
LSVEC-1	-46.030	-26.808	-45.874	-27.051
LSVEC-2	-45.845	-27.129	0.143	0.215
LSVEC	-46.173	-26.569		
LSVEC-1	-46.254	-26.631	-46.196	-26.556
LSVEC-2	-46.162	-26.469	0.050	0.082

Sample UoD

Sample Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS_19	1.179	-0.509		
NBS_19-1	1.551	-0.637	1.308	-0.557
NBS_19-2	1.194	-0.525	0.211	0.070
NBS_19	1.178	-0.111		
NBS_19-1	1.149	-0.445	1.444	-0.149
NBS_19-2	2.005	0.108	0.486	0.278
UoD_1a	-15.846	-1.672		
UoD_1a-1	-14.746	-1.646	-15.378	-1.775
UoD_1a-2	-15.542	-2.008	0.568	0.202
UoD_1b	-15.964	-1.660		
UoD_1b-1	-15.780	-2.297	-15.787	-2.062
UoD_1b-2	-15.617	-2.230	0.174	0.350
UoD_2a	-15.607	-3.187		
UoD_2a-1	-15.503	-3.665	-15.512	-3.508
UoD_2a-2	-15.425	-3.672	0.091	0.278
UoD_3a	-15.984	-3.657		
UoD_3a-1	-15.776	-4.230	-15.873	-3.659
UoD_3a-2	-15.859	-3.090	0.105	0.570
UoD_4a	-15.704	-3.540		
UoD_4a-1	-15.438	-4.619	-15.638	-4.089

UoD_4a-2	-15.771	-4.107	0.176	0.540
UoD_5a	-15.550	-4.394		
UoD_5a-1	-15.639	-4.574	-15.494	-4.546
UoD_5a-2	-15.293	-4.669	0.180	0.140
UoD_6a	-15.720	-5.154		
UoD_6a-1	-15.693	-5.217	-15.680	-5.087
UoD_6a-2	-15.627	-4.889	0.048	0.174
bicarb_X	-4.934	-12.012		
bicarb_X-1	-4.929	-11.764	-4.878	-12.000
bicarb_X-2	-4.772	-12.224	0.092	0.230
bicarb_X	-4.990	-11.489		
bicarb_X-1	-4.616	-11.974	-4.922	-11.733
bicarb_X-2	-5.159	-11.736	0.278	0.243
UoD_7a	-15.553	-4.608		
UoD_7a-1	-15.578	-4.762	-15.570	-4.684
UoD_7a-2	-15.579	-4.682	0.015	0.077
UoD_8a	-15.816	-4.235		
UoD_8a-1	-15.771	-4.746	-15.708	-4.525
UoD_8a-2	-15.538	-4.594	0.149	0.262
UoD_9a	-15.900	-4.982		
UoD_9a-1	-15.704	-5.527	-15.765	-5.300
UoD_9a-2	-15.692	-5.391	0.117	0.284
UoD_10a	-15.568	-5.382		
UoD_10a-1	-15.618	-5.677	-15.530	-5.265
UoD_10a-2	-15.404	-4.735	0.112	0.482
UoD_11a	-15.623	-5.684		
UoD_11a-1	-15.751	-5.410	-15.589	-5.353
UoD_11a-2	-15.394	-4.965	0.181	0.363
UoD_12a	-15.865	-5.550		
UoD_12a-1	-15.688	-4.616	-15.776	-5.126
UoD_12a-2	-15.776	-5.213	0.089	0.473
UoD_13a	-15.652	-4.344		
UoD_13a-1	-15.656	-5.330	-15.640	-4.554
UoD_13a-2	-15.613	-3.987	0.024	0.696
UoD_14a	-16.076	-5.988		
UoD_14a-1	-15.833	-5.745	-15.766	-5.548
UoD_14a-2	-15.389	-4.910	0.348	0.565
UoD_15a	-16.154	-3.037		
UoD_15a-1	-16.030	-3.296	-16.061	-3.216
UoD_15a-2	-16.000	-3.316	0.082	0.156
LSVEC	-46.697	-27.449		
LSVEC-1	-46.502	-27.773	-46.560	-27.570
LSVEC-2	-46.482	-27.487	0.119	0.177
LSVEC	-46.710	-27.453		
LSVEC-1	-46.823	-27.251	-46.759	-27.259
LSVEC-2	-46.744	-27.072	0.058	0.191

Sample X65D10 holes 1-6

Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS19	0.477	-3.327		
NBS19-1	0.366	-3.245	0.480	-3.220
NBS19-2	0.598	-3.088	0.116	0.121
NBS19	0.428	-3.558		
NBS19-1	0.492	-3.587	0.421	-3.516
NBS19-2	0.343	-3.402	0.075	0.100
X65_D10_1a	-16.021	-5.754		
X65_D10_1a-1	-16.328	-6.166	-16.210	-5.878
X65_D10_1a-2	-16.280	-5.715	0.165	0.250
X65_D10_1b	-15.619	-5.786		
X65_D10_1b-1	-15.660	-5.170	-15.632	-5.273
X65_D10_1b-2	-15.618	-4.863	0.024	0.470
X65_D10_2a	-15.546	-5.910		
X65_D10_2a-1	-15.120	-5.721	-15.337	-5.574
X65_D10_2a-2	-15.346	-5.090	0.213	0.429
X65_D10_2b	-15.020	-6.295		
X65_D10_2b-1	-14.855	-6.023	-14.977	-6.291
X65_D10_2b-2	-15.055	-6.556	0.107	0.267
X65_D10_3a	-15.107	-6.203		
X65_D10_3a-1	-15.659	-5.721	-15.209	-5.903
X65_D10_3a-2	-14.861	-5.784	0.409	0.262
X65_D10_3b	-13.861	-6.910		
X65_D10_3b-1	-14.254	-6.133	-14.148	-6.395
X65_D10_3b-2	-14.329	-6.141	0.251	0.446
bicarb-x	-5.550	-13.416		
bicarb-x-1	-5.382	-13.193	-5.468	-13.209
bicarb-x-2	-5.473	-13.017	0.084	0.200
bicarb-x	-5.840	-13.091		
bicarb-x-1	-5.782	-12.862	-5.863	-12.894
bicarb-x-2	-5.968	-12.729	0.095	0.183
X65_D10_4a	-15.830	-4.807		
X65_D10_4a-1	-15.970	-4.177	-15.855	-4.840
X65_D10_4a-2	-15.764	-5.537	0.105	0.681
X65_D10_4b	-15.016	-5.324		
X65_D10_4b-1	-15.795	-4.372	-15.474	-4.513
X65_D10_4b-2	-15.611	-3.842	0.407	0.751
X65_D10_5a	-15.743	-6.463		
X65_D10_5a-1	-15.957	-6.337	-15.943	-6.557
X65_D10_5a-2	-16.130	-6.872	0.194	0.280
X65_D10_5b	-15.682	-6.617		
X65_D10_5b-1	-15.690	-5.838	-15.636	-6.067
X65_D10_5b-2	-15.536	-5.747	0.087	0.478
X65_D10_6a	-14.766	-7.349		
X65_D10_6a-1	-14.837	-6.610	-14.813	-7.166
X65_D10_6a-2	-14.836	-7.540	0.041	0.491
X65_D10_6b	-14.837	-6.999		
X65_D10_6b-1	-15.235	-6.726	-15.033	-6.885

X65_D10_6b-2	-15.028	-6.931	0.199	0.142
LSVEC	-46.370	-27.544		
LSVEC-1	-46.547	-27.131	-46.503	-27.415
LSVEC-2	-46.592	-27.571	0.117	0.247
LSVEC	-46.517	-27.428		
LSVEC-1	-46.582	-27.430	-46.585	-27.725
LSVEC-2	-46.657	-28.318	0.070	0.513

Sample X65D10 holes 7-18

Sample Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS_19	1.250	-0.012		
NBS_19-1	1.129	-0.309	1.242	-0.232
NBS_19-2	1.347	-0.376	0.109	0.194
NBS_19	1.102	0.713		
NBS_19-1	1.287	-0.066	1.223	0.213
NBS_19-2	1.279	-0.009	0.105	0.434
X65_D10_7a	-15.202	-0.239		
X65_D10_7a-1	-14.830	0.386	-15.031	-0.493
X65_D10_7a-2	-15.060	-1.625	0.188	1.029
X65_D10_8a	-14.450	-2.758		
X65_D10_8a-1	-14.241	-2.746	-14.403	-2.785
X65_D10_8a-2	-14.519	-2.851	0.145	0.057
X65_D10_8b	-15.278	-1.785		
X65_D10_8b-1	-14.610	-1.282	-15.189	-1.934
X65_D10_8b-2	-15.680	-2.735	0.540	0.738
X65_D10_9a	-14.630	-1.616		
X65_D10_9a-1	-14.424	-2.473	-14.598	-2.221
X65_D10_9a-2	-14.740	-2.575	0.160	0.527
X65_D10_9b	-15.440	-4.054		
X65_D10_9b-1	-15.562	-3.332	-15.558	-3.733
X65_D10_9b-2	-15.672	-3.812	0.116	0.367
X65_D10_10a	-14.713	-2.815		
X65_D10_10a-1	-14.970	-3.868	-15.064	-3.429
X65_D10_10a-2	-15.510	-3.603	0.407	0.548
X65_D10_10b	-15.291	-3.481		
X65_D10_10b-1	-15.642	-3.305	-15.490	-3.410
X65_D10_10b-2	-15.538	-3.444	0.180	0.093
X65_D10_11a	-15.606	-3.037		
X65_D10_11a-1	-14.877	-4.210	-15.275	-3.789
X65_D10_11a-2	-15.343	-4.120	0.369	0.653
X65_D10_11b	-15.433	-2.274		
X65_D10_11b-1	-15.296	-0.696	-15.228	-1.510
X65_D10_11b-2	-14.954	-1.559	0.247	0.790
X65_D10_12a	-14.997	-2.555		
X65_D10_12a-1	-15.216	-2.787	-15.161	-2.855
X65_D10_12a-2	-15.269	-3.223	0.144	0.339

X65_D10_12b	-14.511	-5.893		
X65_D10_12b-1	-14.661	-5.911	-14.630	-5.923
X65_D10_12b-2	-14.719	-5.964	0.107	0.037
Bicarb_X	-5.091	-13.091		
Bicarb_X-1	-5.225	-12.534	-5.129	-12.774
Bicarb_X-2	-5.071	-12.697	0.084	0.286
Bicarb_X	-5.201	-12.873		
Bicarb_X-1	-5.232	-12.807	-5.229	-12.535
Bicarb_X-2	-5.254	-11.924	0.027	0.530
X65_D10_13a	-15.342	-5.431		
X65_D10_13a-1	-15.201	-4.342	-15.342	-4.849
X65_D10_13a-2	-15.484	-4.775	0.142	0.548
X65_D10_13b	-14.116	-5.694		
X65_D10_13b-1	-13.529	-5.321	-13.826	-5.379
X65_D10_13b-2	-13.834	-5.123	0.294	0.290
X65_D10_14a	-14.864	-2.969		
X65_D10_14a-1	-15.000	-2.910	-14.949	-3.062
X65_D10_14a-2	-14.984	-3.308	0.074	0.215
X65_D10_14b	-15.445	-4.348		
X65_D10_14b-1	-15.646	-3.778	-15.455	-4.040
X65_D10_14b-2	-15.274	-3.995	0.186	0.288
X65_D10_15a	-14.324	-4.824		
X65_D10_15a-1	-14.410	-6.109	-14.349	-5.670
X65_D10_15a-2	-14.314	-6.076	0.053	0.733
X65_D10_15b	-14.309	-4.183		
X65_D10_15b-1	-14.339	-4.407	-14.283	-4.331
X65_D10_15b-2	-14.201	-4.402	0.073	0.128
X65_D10_16a	-14.895	-5.230		
X65_D10_16a-1	-15.389	-5.981	-15.258	-5.623
X65_D10_16a-2	-15.489	-5.658	0.318	0.377
X65_D10_16b	-15.270	-4.529		
X65_D10_16b-1	-15.840	-4.729	-15.867	-3.892
X65_D10_16b-2	-16.490	-2.418	0.610	1.280
X65_D10_17a	-14.209	-1.070		
X65_D10_17a-1	-14.473	-4.052	-14.495	-4.355
X65_D10_17a-2	-14.517	-4.657	0.031	0.428
X65_D10_17b	-14.428	-4.068		
X65_D10_17b-1	-14.434	-4.583	-14.474	-4.297
X65_D10_17b-2	-14.561	-4.239	0.075	0.262
X65_D10_18a	-13.972	-4.990		
X65_D10_18a-1	-13.822	-5.130	-14.004	-5.107
X65_D10_18a-2	-14.217	-5.200	0.199	0.107
X65_D10_18b	-14.253	-2.751		
X65_D10_18b-1	-14.384	-2.871	-14.354	-2.758
X65_D10_18b-2	-14.426	-2.653	0.090	0.109
LSVEC	-46.686	-27.212		
LSVEC-1	-46.780	-27.360	-46.717	-27.235
LSVEC-2	-46.684	-27.132	0.055	0.116

LSVEC	-46.201	-27.404		
LSVEC-1	-46.287	-27.345	-46.270	-27.399
LSVEC-2	-46.323	-27.448	0.063	0.052

Sample JR3_14

Sample Name	^{13}C	^{18}O	Average SD ^{13}C	Average SD ^{18}O
NBS19	0.855	-1.077		
NBS19-1	0.746	-1.379	0.750	-1.547
NBS19-2	0.649	-2.184	0.103	0.572
NBS19	0.555	-1.722		
NBS19-1	0.694	-1.753	0.623	-1.751
NBS19-2	0.620	-1.778	0.070	0.028
JR3_14_1a	-13.862	-3.650		
JR3_14_1a-1	-13.941	-3.702	-13.945	-3.273
JR3_14_1a-2	-14.033	-2.466	0.086	0.699
JR3_14_1b	-13.525	-4.196		
JR3_14_1b-1	-13.473	-4.399	-13.434	-4.460
JR3_14_1b-2	-13.305	-4.784	0.115	0.299
JR3_14_2a	-13.880	-3.477		
JR3_14_2a-1	-13.754	-4.044	-13.917	-3.744
JR3_14_2a-2	-14.118	-3.712	0.185	0.285
JR_3_14_2b	-13.321	-4.893		
JR_3_14_2b-1	-13.840	-4.526	-13.499	-4.254
JR_3_14_2b-2	-13.337	-3.343	0.295	0.810
JR_3_14_3a	-13.656	-5.403		
JR_3_14_3a-1	-13.779	-5.482	-13.693	-5.387
JR_3_14_3a-2	-13.645	-5.275	0.074	0.104
JR_3_14_3b	-13.785	-5.386		
JR_3_14_3b-1	-13.777	-4.795	-13.794	-4.932
JR_3_14_3b-2	-13.819	-4.614	0.022	0.404
JR_3_14_4a	-14.008	-4.014		
JR_3_14_4a-1	-14.155	-4.771	-13.978	-4.436
JR_3_14_4a-2	-13.770	-4.523	0.194	0.386
JR_3_14_4b	-14.215	-5.755		
JR_3_14_4b-1	-14.121	-4.745	-14.151	-5.550
JR_3_14_4b-2	-14.116	-6.151	0.056	0.725
bicarb-x	-5.615	-12.056		
bicarb-x-1	-5.434	-12.718	-5.498	-12.143
bicarb-x-2	-5.444	-11.654	0.102	0.537
bicarb-x	-5.757	-12.785		
bicarb-x-1	-5.874	-13.014	-5.805	-12.824
bicarb-x-2	-5.783	-12.674	0.061	0.173
JR_3_14_5a	-13.369	-6.326		
JR_3_14_5a-1	-13.435	-5.916	-13.337	-6.010
JR_3_14_5a-2	-13.208	-5.787	0.117	0.281
JR_3_14_5b	-12.997	-5.732		
JR_3_14_5b-1	-12.625	-5.520	-12.860	-5.709

JR_3_14_5b-2	-12.959	-5.874	0.205	0.178
JR_3_14_6a	-13.566	-4.524		
JR_3_14_6a-1	-13.537	-5.702	-13.705	-4.970
JR_3_14_6a-2	-14.011	-4.685	0.266	0.639
JR_3_14_6b	-13.664	-6.279		
JR_3_14_6b-1	-13.197	-5.686	-13.371	-5.932
JR_3_14_6b-2	-13.251	-5.831	0.255	0.309
JR_3_14_7a	-14.062	-5.720		
JR_3_14_7a-1	-13.932	-5.424	-13.955	-5.646
JR_3_14_7a-2	-13.871	-5.793	0.098	0.195
JR_3_14_7b	-13.653	-6.079		
JR_3_14_7b-1	-13.915	-5.941	-13.717	-6.101
JR_3_14_7b-2	-13.583	-6.282	0.175	0.172
JR_3_14_8a	-14.574	-5.549		
JR_3_14_8a-1	-14.297	-5.962	-14.248	-6.030
JR_3_14_8a-2	-13.872	-6.578	0.354	0.518
JR_3_14_8b	-14.957	-5.767		
JR_3_14_8b-1	-14.782	-4.664	-14.821	-5.617
JR_3_14_8b-2	-14.725	-6.421	0.121	0.888
LSVEC	-45.175	-26.377		
LSVEC-1	-45.101	-26.678	-45.145	-26.568
LSVEC-2	-45.160	-26.650	0.039	0.166
LSVEC	-46.857	-27.722		
LSVEC-1	-46.923	-27.810	-46.888	-27.935
LSVEC-2	-46.885	-28.274	0.033	0.297

Appendix 3: Example of the calculations used to estimate the $\delta^{18}O$ values of drinking water

UoD

Example transfer from $\delta^{18}O$ bone carbonate values (on the VPDB scale) to the likely $\delta^{18}O$ value of drinking water:-

$\delta^{18}O_{\text{carbonate VSMOW}}(x) = 1.03086\ \delta^{18}O_{\text{carbonate VPDB}}(x) + 30.86$
Bone carbonate (V_{SMOW}) = (1.03086*-5.82) + 30.86 = 24.86‰

$\delta^{18}O_{\text{phosphate VSMOW}} = 0.98\ \delta^{18}O_{\text{carbonate}} - 8.5$
Bone phosphate (V_{VSMOW}) = (0.98*24.86) – 8.5 = 15.37‰

$\delta^{18}O$ source water $= 1.54 * \delta^{18}O_{\text{phosphate}} - 33.72$
Likely δ value of drinking water = (1.54*15.37) – 33.72 = -9.30‰

792L

Example transfer from $\delta^{18}O$ bone carbonate values (on the VPDB scale) to the likely $\delta^{18}O$ value of drinking water:-

$\delta^{18}O_{\text{carbonate VSMOW}}(x) = 1.03086\ \delta^{18}O_{\text{carbonate VPDB}}(x) + 30.86$
Bone carbonate (V_{SMOW}) = (1.03086*-3.57) + 30.86 = 27.18‰

$\delta^{18}O_{\text{phosphate VSMOW}} = 0.98\ \delta^{18}O_{\text{carbonate}} - 8.5$
Bone phosphate (V_{VSMOW}) = (0.98*27.18) – 8.5 = 18.14‰

$\delta^{18}O$ source water $= 1.54 * \delta^{18}O_{\text{phosphate}} - 33.72$
Likely δ value of drinking water = (1.54*18.14) – 33.72 = -5.79‰

Appendix 4: One-way ANOVA for the $\delta^{18}O$ values for bone sections 792L and 792R, and 792L, 792R, and 820R.

Group Name	N	Missing	Mean	Std Dev	SEM
792R	48	0	-2.825	0.911	0.131
792L	51	3	-2.977	0.895	0.129

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.553	0.553	0.678	0.412
Residual	94	76.690	0.816		
Total	95	77.243			

Group Name	N	Missing	Mean	Std Dev	SEM
792R	48	0	-2.825	0.911	0.131
792L	51	3	-2.977	0.895	0.129
820R	51	3	-2.922	1.488	0.215

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.568	0.284	0.222	0.802
Residual	141	180.773	1.282		
Total	143	181.341			

Appendix 5: One-way ANOVA for the $\delta^{13}C$ values for 792L and 792R

Group Name	N	Missing	Mean	Std Dev	SEM
792R	48	0	-12.864	0.376	0.0542
792L	48	0	-12.917	0.505	0.0729

Source of Variation	DF	SS	MS	F	P
Between Groups	1	0.0676	0.0676	0.341	0.560
Residual	94	18.624	0.198		
Total	95	18.692			

Appendix 6: One-way ANOVA for the $\delta^{13}C$ values for 820R and X65 D10

Group Name	N	Missing	Mean	Std Dev	SEM
820R	48	0	-14.822	4.503	0.650
X65 D10	105	0	-15.042	0.619	0.0604

Source of Variation	DF	SS	MS	F	P
Between Groups	1	1.591	1.591	0.242	0.623
Residual	151	992.788	6.575		
Total	152	994.380			

Appendix 7: One-way ANOVA for the $\delta^{13}C$ and $\delta^{18}O$ values for KAS2 and RPT

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
KAS2	48	0	-16.343	1.010	0.146
RPT	48	0	-16.983	0.477	0.0688

Source of Variation	DF	SS	MS	F	P
Between Groups	1	9.814	9.814	15.727	<0.001
Residual	94	58.660	0.624		
Total	95	68.474			

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
KAS2	48	0	-6.558	1.312	0.189
RPT	48	0	-6.872	0.850	0.123

Source of Variation	DF	SS	MS	F	P
Between Groups	1	2.365	2.365	1.935	0.167
Residual	94	114.888	1.222		
Total	95	117.254			

Appendix 8: Table of the results of descriptive statistical tests on the standards run with KAS2 and KAS2 RPT

Column	Size	Missing	Mean	Std Dev	Std. Error
NBS-19	4	0	-1.988	0.741	0.370
BICARB-X	4	0	-12.928	0.100	0.0502
LSVEC	4	0	-27.282	0.588	0.294

Appendix 9: Table of the results of descriptive statistical tests on bone section JR_3 14

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	48	0	-14.822	4.503	0.650
Oxygen Value	48	0	-2.922	1.488	0.215

Column	Range	Max	Min	Median
Carbon Value	31.851	15.654	-16.197	-15.461
Oxygen Value	8.270	3.000	-5.270	-2.767

Appendix 10: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section JR_3 14

Dependent Variable: Carbon Value

Hole Number	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-13.690	0.294	0.120
2.000	6	0	-13.708	0.318	0.130
3.000	6	0	-13.743	0.0737	0.0301
4.000	6	0	-14.064	0.159	0.0650
5.000	6	0	-13.099	0.301	0.123
6.000	6	0	-13.538	0.296	0.121
7.000	6	0	-13.836	0.182	0.0742
8.000	6	0	-14.535	0.393	0.161

Source of Variation	DF	SS	MS	F	P
Between Groups	7	7.142	1.020	13.973	<0.001
Residual	40	2.921	0.0730		
Total	47	10.063			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
5.000 vs. 8.000	1.436	9.202	2.007E-011	0.002	Yes
6.000 vs. 8.000	0.997	6.389	0.000000134	0.002	Yes
5.000 vs. 4.000	0.965	6.188	0.000000258	0.002	Yes
1.000 vs. 8.000	0.845	5.414	0.00000314	0.002	Yes
2.000 vs. 8.000	0.826	5.296	0.00000460	0.002	Yes
3.000 vs. 8.000	0.791	5.070	0.00000947	0.002	Yes
5.000 vs. 7.000	0.737	4.725	0.0000283	0.002	Yes
7.000 vs. 8.000	0.699	4.477	0.0000616	0.002	Yes
5.000 vs. 3.000	0.645	4.132	0.000178	0.003	Yes
5.000 vs. 2.000	0.610	3.907	0.000352	0.003	Yes
5.000 vs. 1.000	0.591	3.788	0.000500	0.003	Yes
6.000 vs. 4.000	0.526	3.375	0.00165	0.003	Yes
4.000 vs. 8.000	0.470	3.015	0.00445	0.003	No
5.000 vs. 6.000	0.439	2.813	0.00758	0.003	No
1.000 vs. 4.000	0.374	2.399	0.0212	0.004	No
2.000 vs. 4.000	0.356	2.281	0.0280	0.004	No

3.000 vs. 4.000	0.321	2.055	0.0464	0.004	No
6.000 vs. 7.000	0.298	1.912	0.0630	0.005	No
7.000 vs. 4.000	0.228	1.462	0.151	0.005	No
6.000 vs. 3.000	0.206	1.319	0.195	0.006	No
6.000 vs. 2.000	0.171	1.094	0.281	0.006	No
6.000 vs. 1.000	0.152	0.975	0.335	0.007	No
1.000 vs. 7.000	0.146	0.937	0.354	0.009	No
2.000 vs. 7.000	0.128	0.818	0.418	0.010	No
3.000 vs. 7.000	0.0925	0.593	0.557	0.013	No
1.000 vs. 3.000	0.0537	0.344	0.733	0.017	No
2.000 vs. 3.000	0.0352	0.225	0.823	0.025	No
1.000 vs. 2.000	0.0185	0.119	0.906	0.050	No

Dependent Variable: Oxygen Value

Hole Number	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-3.866	0.809	0.330
2.000	6	0	-3.999	0.611	0.249
3.000	6	0	-5.159	0.363	0.148
4.000	6	0	-4.993	0.801	0.327
5.000	6	0	-5.859	0.268	0.109
6.000	6	0	-5.451	0.692	0.283
7.000	6	0	-5.873	0.299	0.122
8.000	6	0	-5.823	0.688	0.281

Source of Variation	DF	SS	MS	F	P
Between Groups	7	27.382	3.912	10.739	<0.001
Residual	40	14.570	0.364		
Total	47	41.952			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 7.000	2.007	5.760	0.00000103	0.002	Yes
1.000 vs. 5.000	1.993	5.720	0.00000117	0.002	Yes
1.000 vs. 8.000	1.957	5.617	0.00000163	0.002	Yes
2.000 vs. 7.000	1.874	5.378	0.00000352	0.002	Yes
2.000 vs. 5.000	1.860	5.338	0.00000401	0.002	Yes

2.000 vs. 8.000	1.824	5.236	0.00000557	0.002	Yes
1.000 vs. 6.000	1.585	4.549	0.0000492	0.002	Yes
2.000 vs. 6.000	1.452	4.167	0.000160	0.002	Yes
1.000 vs. 3.000	1.293	3.711	0.000628	0.003	Yes
2.000 vs. 3.000	1.160	3.329	0.00188	0.003	Yes
1.000 vs. 4.000	1.127	3.234	0.00245	0.003	Yes
2.000 vs. 4.000	0.994	2.853	0.00683	0.003	No
4.000 vs. 7.000	0.880	2.525	0.0156	0.003	No
4.000 vs. 5.000	0.866	2.485	0.0172	0.003	No
4.000 vs. 8.000	0.830	2.383	0.0220	0.004	No
3.000 vs. 7.000	0.714	2.049	0.0471	0.004	No
3.000 vs. 5.000	0.700	2.009	0.0513	0.004	No
3.000 vs. 8.000	0.664	1.907	0.0638	0.005	No
4.000 vs. 6.000	0.458	1.314	0.196	0.005	No
6.000 vs. 7.000	0.422	1.211	0.233	0.006	No
6.000 vs. 5.000	0.408	1.171	0.249	0.006	No
6.000 vs. 8.000	0.372	1.069	0.292	0.007	No
3.000 vs. 6.000	0.292	0.838	0.407	0.009	No
4.000 vs. 3.000	0.166	0.476	0.636	0.010	No
1.000 vs. 2.000	0.133	0.382	0.705	0.013	No
8.000 vs. 7.000	0.0497	0.143	0.887	0.017	No
8.000 vs. 5.000	0.0357	0.102	0.919	0.025	No
5.000 vs. 7.000	0.0140	0.0402	0.968	0.050	No

Appendix 11: Table of the results of descriptive statistical tests on bone section X65 D10

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	105	0	-15.042	0.619	0.0604

Oxygen Value	105	0	-4.448	1.693	0.165

Column	Range	Max	Min	Median
Carbon Value	2.961	-13.529	-16.490	-15.028
Oxygen Value	7.926	0.386	-7.540	-4.583

Appendix 12: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section X65 D10

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-15.921	0.333	0.136
2.000	6	0	-15.157	0.249	0.101
3.000	6	0	-14.679	0.656	0.268
4.000	6	0	-15.664	0.338	0.138
5.000	6	0	-15.790	0.215	0.0879
6.000	6	0	-14.923	0.176	0.0720
7.000	3	0	-15.031	0.188	0.108
8.000	6	0	-14.796	0.557	0.228
9.000	6	0	-15.078	0.541	0.221
10.000	6	0	-15.277	0.366	0.149
11.000	6	0	-15.252	0.282	0.115
12.000	6	0	-14.895	0.312	0.127
13.000	6	0	-14.584	0.856	0.349
14.000	6	0	-15.202	0.305	0.124
15.000	6	0	-14.316	0.0674	0.0275
16.000	6	0	-15.562	0.548	0.224
17.000	6	0	-14.437	0.123	0.0500
18.000	6	0	-14.179	0.237	0.0967

Source of Variation	DF	SS	MS	F	P
Between Groups	17	25.225	1.484	8.834	<0.001
Residual	87	14.614	0.168		
Total	104	39.839			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
18.000 vs. 1.000	1.742	7.362	9.619E-011	0.000	Yes
18.000 vs. 5.000	1.611	6.807	0.00000000122	0.000	Yes
15.000 vs. 1.000	1.605	6.782	0.00000000136	0.000	Yes
18.000 vs. 4.000	1.485	6.277	0.0000000131	0.000	Yes
17.000 vs. 1.000	1.484	6.271	0.0000000134	0.000	Yes
15.000 vs. 5.000	1.474	6.227	0.0000000163	0.000	Yes
18.000 vs. 16.000	1.383	5.845	0.0000000863	0.000	Yes
17.000 vs. 5.000	1.353	5.716	0.000000150	0.000	Yes
15.000 vs.	1.348	5.697	0.000000163	0.000	Yes

4.000					
13.000 vs. 1.000	1.337	5.649	0.000000200	0.000	Yes
15.000 vs. 16.000	1.246	5.266	0.000000997	0.000	Yes
3.000 vs. 1.000	1.242	5.251	0.00000106	0.000	Yes
17.000 vs. 4.000	1.227	5.187	0.00000138	0.000	Yes
13.000 vs. 5.000	1.205	5.094	0.00000202	0.000	Yes
17.000 vs. 16.000	1.125	4.755	0.00000780	0.000	Yes
8.000 vs. 1.000	1.125	4.753	0.00000787	0.000	Yes
3.000 vs. 5.000	1.111	4.696	0.00000983	0.000	Yes
18.000 vs. 10.000	1.098	4.642	0.0000121	0.000	Yes
13.000 vs. 4.000	1.080	4.564	0.0000164	0.000	Yes
18.000 vs. 11.000	1.073	4.532	0.0000185	0.000	Yes
12.000 vs. 1.000	1.026	4.334	0.0000392	0.000	Yes
18.000 vs. 14.000	1.023	4.324	0.0000407	0.000	Yes
6.000 vs. 1.000	0.998	4.217	0.0000604	0.000	Yes
8.000 vs. 5.000	0.993	4.198	0.0000648	0.000	Yes
3.000 vs. 4.000	0.986	4.166	0.0000728	0.000	Yes
18.000 vs. 2.000	0.978	4.133	0.0000821	0.000	Yes
13.000 vs. 16.000	0.978	4.132	0.0000823	0.000	Yes
15.000 vs. 10.000	0.961	4.062	0.000106	0.000	Yes
15.000 vs. 11.000	0.935	3.953	0.000157	0.000	Yes
18.000 vs. 9.000	0.899	3.799	0.000268	0.000	Yes
12.000 vs. 5.000	0.894	3.779	0.000288	0.000	Yes
15.000 vs. 14.000	0.886	3.744	0.000324	0.000	Yes
3.000 vs. 16.000	0.884	3.734	0.000335	0.000	Yes
8.000 vs. 4.000	0.868	3.668	0.000420	0.000	Yes
6.000 vs. 5.000	0.867	3.662	0.000429	0.000	Yes
9.000 vs. 1.000	0.843	3.563	0.000599	0.000	No

15.000 vs. 2.000	0.841	3.553	0.000617	0.000	No
17.000 vs. 10.000	0.840	3.551	0.000621	0.000	No
17.000 vs. 11.000	0.815	3.442	0.000889	0.000	No
12.000 vs. 4.000	0.769	3.249	0.00165	0.000	No
8.000 vs. 16.000	0.766	3.236	0.00171	0.000	No
17.000 vs. 14.000	0.765	3.234	0.00173	0.000	No
2.000 vs. 1.000	0.764	3.229	0.00175	0.000	No
15.000 vs. 9.000	0.762	3.220	0.00181	0.000	No
18.000 vs. 6.000	0.744	3.145	0.00227	0.000	No
6.000 vs. 4.000	0.741	3.132	0.00236	0.000	No
7.000 vs. 1.000	0.890	3.072	0.00284	0.000	No
17.000 vs. 2.000	0.720	3.043	0.00310	0.000	No
14.000 vs. 1.000	0.719	3.038	0.00315	0.000	No
18.000 vs. 12.000	0.716	3.028	0.00324	0.000	No
9.000 vs. 5.000	0.712	3.008	0.00344	0.000	No
18.000 vs. 7.000	0.852	2.939	0.00422	0.001	No
13.000 vs. 10.000	0.693	2.929	0.00435	0.001	No
11.000 vs. 1.000	0.669	2.829	0.00579	0.001	No
13.000 vs. 11.000	0.667	2.819	0.00596	0.001	No
12.000 vs. 16.000	0.667	2.817	0.00599	0.001	No
10.000 vs. 1.000	0.644	2.720	0.00788	0.001	No
17.000 vs. 9.000	0.641	2.709	0.00813	0.001	No
6.000 vs. 16.000	0.639	2.700	0.00832	0.001	No
2.000 vs. 5.000	0.633	2.674	0.00896	0.001	No
7.000 vs. 5.000	0.759	2.619	0.0104	0.001	No
13.000 vs. 14.000	0.618	2.611	0.0106	0.001	No
18.000 vs. 8.000	0.617	2.609	0.0107	0.001	No
15.000 vs.	0.607	2.565	0.0120	0.001	No

6.000					
3.000 vs. 10.000	0.599	2.531	0.0132	0.001	No
14.000 vs. 5.000	0.588	2.483	0.0150	0.001	No
9.000 vs. 4.000	0.586	2.478	0.0151	0.001	No
15.000 vs. 7.000	0.715	2.465	0.0156	0.001	No
15.000 vs. 12.000	0.579	2.448	0.0164	0.001	No
3.000 vs. 11.000	0.573	2.422	0.0175	0.001	No
13.000 vs. 2.000	0.573	2.420	0.0176	0.001	No
11.000 vs. 5.000	0.538	2.274	0.0254	0.001	No
3.000 vs. 14.000	0.524	2.213	0.0295	0.001	No
7.000 vs. 4.000	0.634	2.187	0.0315	0.001	No
10.000 vs. 5.000	0.512	2.165	0.0331	0.001	No
2.000 vs. 4.000	0.507	2.144	0.0348	0.001	No
18.000 vs. 3.000	0.499	2.111	0.0376	0.001	No
13.000 vs. 9.000	0.494	2.086	0.0399	0.001	No
17.000 vs. 6.000	0.486	2.055	0.0429	0.001	No
17.000 vs. 7.000	0.594	2.048	0.0435	0.001	No
9.000 vs. 16.000	0.484	2.046	0.0438	0.001	No
8.000 vs. 10.000	0.481	2.033	0.0451	0.001	No
15.000 vs. 8.000	0.480	2.029	0.0455	0.001	No
3.000 vs. 2.000	0.479	2.022	0.0462	0.001	No
14.000 vs. 4.000	0.462	1.953	0.0540	0.001	No
17.000 vs. 12.000	0.459	1.938	0.0559	0.001	No
8.000 vs. 11.000	0.455	1.924	0.0577	0.001	No
7.000 vs. 16.000	0.531	1.834	0.0701	0.001	No
11.000 vs. 4.000	0.413	1.745	0.0846	0.001	No
8.000 vs. 14.000	0.406	1.715	0.0899	0.001	No
18.000 vs. 13.000	0.405	1.713	0.0903	0.001	No

2.000 vs. 16.000	0.405	1.712	0.0904	0.001	No
3.000 vs. 9.000	0.400	1.688	0.0949	0.001	No
10.000 vs. 4.000	0.387	1.635	0.106	0.001	No
12.000 vs. 10.000	0.382	1.614	0.110	0.001	No
13.000 vs. 7.000	0.446	1.540	0.127	0.001	No
15.000 vs. 3.000	0.362	1.531	0.129	0.001	No
8.000 vs. 2.000	0.361	1.524	0.131	0.001	No
14.000 vs. 16.000	0.360	1.521	0.132	0.001	No
17.000 vs. 8.000	0.359	1.519	0.132	0.001	No
16.000 vs. 1.000	0.359	1.516	0.133	0.001	No
12.000 vs. 11.000	0.356	1.504	0.136	0.001	No
6.000 vs. 10.000	0.354	1.497	0.138	0.001	No
13.000 vs. 6.000	0.339	1.432	0.156	0.001	No
6.000 vs. 11.000	0.328	1.388	0.169	0.001	No
13.000 vs. 12.000	0.311	1.315	0.192	0.001	No
11.000 vs. 16.000	0.311	1.313	0.193	0.001	No
12.000 vs. 14.000	0.307	1.296	0.198	0.001	No
3.000 vs. 7.000	0.352	1.215	0.228	0.001	No
10.000 vs. 16.000	0.285	1.204	0.232	0.001	No
8.000 vs. 9.000	0.282	1.190	0.237	0.001	No
6.000 vs. 14.000	0.279	1.179	0.242	0.001	No
15.000 vs. 13.000	0.268	1.133	0.260	0.001	No
12.000 vs. 2.000	0.262	1.105	0.272	0.001	No
18.000 vs. 17.000	0.258	1.090	0.279	0.001	No
4.000 vs. 1.000	0.257	1.085	0.281	0.001	No
3.000 vs. 6.000	0.245	1.034	0.304	0.001	No
17.000 vs. 3.000	0.242	1.021	0.310	0.001	No
6.000 vs.	0.234	0.988	0.326	0.001	No

2.000					
16.000 vs. 5.000	0.228	0.961	0.339	0.002	No
3.000 vs. 12.000	0.217	0.917	0.362	0.002	No
13.000 vs. 8.000	0.212	0.896	0.373	0.002	No
7.000 vs. 10.000	0.247	0.851	0.397	0.002	No
9.000 vs. 10.000	0.199	0.842	0.402	0.002	No
8.000 vs. 7.000	0.234	0.809	0.421	0.002	No
12.000 vs. 9.000	0.183	0.771	0.443	0.002	No
7.000 vs. 11.000	0.221	0.762	0.448	0.002	No
9.000 vs. 11.000	0.174	0.733	0.465	0.002	No
6.000 vs. 9.000	0.155	0.654	0.515	0.002	No
17.000 vs. 13.000	0.147	0.623	0.535	0.002	No
7.000 vs. 14.000	0.171	0.592	0.556	0.002	No
18.000 vs. 15.000	0.137	0.580	0.564	0.002	No
5.000 vs. 1.000	0.131	0.555	0.580	0.002	No
8.000 vs. 6.000	0.127	0.536	0.593	0.003	No
4.000 vs. 5.000	0.125	0.530	0.598	0.003	No
9.000 vs. 14.000	0.124	0.525	0.601	0.003	No
15.000 vs. 17.000	0.121	0.511	0.611	0.003	No
2.000 vs. 10.000	0.120	0.509	0.612	0.003	No
3.000 vs. 8.000	0.118	0.498	0.620	0.003	No
12.000 vs. 7.000	0.135	0.466	0.642	0.004	No
7.000 vs. 2.000	0.126	0.436	0.664	0.004	No
16.000 vs. 4.000	0.102	0.432	0.667	0.004	No
8.000 vs. 12.000	0.0992	0.419	0.676	0.005	No
2.000 vs. 11.000	0.0945	0.399	0.691	0.005	No
13.000 vs. 3.000	0.0942	0.398	0.692	0.006	No
6.000 vs. 7.000	0.107	0.371	0.712	0.006	No

9.000 vs. 2.000	0.0790	0.334	0.739	0.007	No
14.000 vs. 10.000	0.0752	0.318	0.752	0.009	No
14.000 vs. 11.000	0.0493	0.208	0.835	0.010	No
2.000 vs. 14.000	0.0452	0.191	0.849	0.013	No
7.000 vs. 9.000	0.0473	0.163	0.871	0.017	No
12.000 vs. 6.000	0.0277	0.117	0.907	0.025	No
11.000 vs. 10.000	0.0258	0.109	0.913	0.050	No

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-5.576	0.473	0.193
2.000	6	0	-5.933	0.507	0.207
3.000	6	0	-6.149	0.424	0.173
4.000	6	0	-4.676	0.666	0.272
5.000	6	0	-6.312	0.441	0.180
6.000	6	0	-7.026	0.358	0.146
7.000	3	0	-0.493	1.029	0.594
8.000	6	0	-2.360	0.661	0.270
9.000	6	0	-2.977	0.922	0.376
10.000	6	0	-3.419	0.351	0.143
11.000	6	0	-2.649	1.407	0.574
12.000	6	0	-4.389	1.694	0.692
13.000	6	0	-5.114	0.488	0.199
14.000	6	0	-3.551	0.582	0.238
15.000	6	0	-5.000	0.871	0.356
16.000	6	0	-4.757	1.269	0.518
17.000	6	0	-3.778	1.351	0.552
18.000	6	0	-3.932	1.290	0.527

Source of Variation	DF	SS	MS	F	P
Between Groups	17	225.293	13.253	15.855	<0.001
Residual	87	72.722	0.836		
Total	104	298.014			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
7.000 vs. 6.000	6.533	10.106	2.461E-016	0.000	Yes
7.000 vs. 5.000	5.820	9.002	4.448E-014	0.000	Yes
8.000 vs. 6.000	4.666	8.840	9.539E-014	0.000	Yes
7.000 vs. 3.000	5.656	8.749	1.467E-013	0.000	Yes
7.000 vs. 2.000	5.440	8.415	7.081E-013	0.000	Yes
11.000 vs.	4.376	8.291	1.264E-012	0.000	Yes

6.000					
7.000 vs. 1.000	5.083	7.863	9.407E-012	0.000	Yes
9.000 vs. 6.000	4.049	7.670	2.302E-011	0.000	Yes
8.000 vs. 5.000	3.953	7.489	5.354E-011	0.000	Yes
8.000 vs. 3.000	3.789	7.178	0.000000000224	0.000	Yes
7.000 vs. 13.000	4.622	7.149	0.000000000256	0.000	Yes
7.000 vs. 15.000	4.508	6.972	0.000000000574	0.000	Yes
11.000 vs. 5.000	3.663	6.939	0.000000000667	0.000	Yes
10.000 vs. 6.000	3.606	6.832	0.00000000109	0.000	Yes
8.000 vs. 2.000	3.573	6.769	0.00000000145	0.000	Yes
11.000 vs. 3.000	3.499	6.629	0.00000000272	0.000	Yes
7.000 vs. 16.000	4.265	6.597	0.00000000314	0.000	Yes
14.000 vs. 6.000	3.474	6.582	0.00000000336	0.000	Yes
7.000 vs. 4.000	4.184	6.472	0.00000000551	0.000	Yes
9.000 vs. 5.000	3.335	6.319	0.0000000109	0.000	Yes
11.000 vs. 2.000	3.283	6.220	0.0000000168	0.000	Yes
17.000 vs. 6.000	3.248	6.153	0.0000000226	0.000	Yes
8.000 vs. 1.000	3.216	6.093	0.0000000294	0.000	Yes
7.000 vs. 12.000	3.896	6.027	0.0000000393	0.000	Yes
9.000 vs. 3.000	3.172	6.009	0.0000000425	0.000	Yes
18.000 vs. 6.000	3.093	5.860	0.0000000809	0.000	Yes
9.000 vs. 2.000	2.955	5.599	0.000000247	0.000	Yes
11.000 vs. 1.000	2.926	5.544	0.000000312	0.000	Yes
10.000 vs. 5.000	2.893	5.481	0.000000407	0.000	Yes
7.000 vs. 18.000	3.440	5.321	0.000000794	0.000	Yes
14.000 vs. 5.000	2.761	5.231	0.00000115	0.000	Yes
8.000 vs. 13.000	2.755	5.219	0.00000121	0.000	Yes
10.000 vs. 3.000	2.729	5.171	0.00000147	0.000	Yes

7.000 vs. 17.000	3.286	5.082	0.00000211	0.000	Yes
8.000 vs. 15.000	2.641	5.003	0.00000291	0.000	Yes
12.000 vs. 6.000	2.637	4.996	0.00000300	0.000	Yes
9.000 vs. 1.000	2.599	4.923	0.00000401	0.000	Yes
14.000 vs. 3.000	2.597	4.921	0.00000405	0.000	Yes
17.000 vs. 5.000	2.534	4.801	0.00000651	0.000	Yes
10.000 vs. 2.000	2.513	4.761	0.00000762	0.000	Yes
7.000 vs. 14.000	3.059	4.731	0.00000856	0.000	Yes
11.000 vs. 13.000	2.465	4.670	0.0000109	0.000	Yes
8.000 vs. 16.000	2.398	4.543	0.0000178	0.000	Yes
7.000 vs. 10.000	2.927	4.527	0.0000189	0.000	Yes
14.000 vs. 2.000	2.381	4.511	0.0000201	0.000	Yes
18.000 vs. 5.000	2.380	4.509	0.0000203	0.000	Yes
17.000 vs. 3.000	2.370	4.491	0.0000217	0.000	Yes
11.000 vs. 15.000	2.351	4.454	0.0000250	0.000	Yes
4.000 vs. 6.000	2.349	4.451	0.0000252	0.000	Yes
8.000 vs. 4.000	2.317	4.389	0.0000318	0.000	Yes
16.000 vs. 6.000	2.268	4.297	0.0000449	0.000	Yes
18.000 vs. 3.000	2.216	4.198	0.0000647	0.001	Yes
10.000 vs. 1.000	2.156	4.085	0.0000977	0.001	Yes
17.000 vs. 2.000	2.154	4.081	0.0000990	0.001	Yes
9.000 vs. 13.000	2.137	4.049	0.000111	0.001	Yes
11.000 vs. 16.000	2.108	3.994	0.000136	0.001	Yes
8.000 vs. 12.000	2.029	3.845	0.000229	0.001	Yes
7.000 vs. 9.000	2.484	3.843	0.000231	0.001	Yes
11.000 vs. 4.000	2.027	3.840	0.000233	0.001	Yes
15.000 vs. 6.000	2.026	3.838	0.000235	0.001	Yes
14.000 vs.	2.024	3.835	0.000237	0.001	Yes

1.000					
9.000 vs. 15.000	2.023	3.833	0.000239	0.001	Yes
18.000 vs. 2.000	2.000	3.789	0.000278	0.001	Yes
12.000 vs. 5.000	1.924	3.644	0.000456	0.001	Yes
13.000 vs. 6.000	1.911	3.621	0.000492	0.001	Yes
17.000 vs. 1.000	1.797	3.405	0.00100	0.001	No
9.000 vs. 16.000	1.780	3.373	0.00111	0.001	No
7.000 vs. 11.000	2.157	3.336	0.00125	0.001	No
12.000 vs. 3.000	1.760	3.334	0.00126	0.001	No
11.000 vs. 12.000	1.740	3.295	0.00142	0.001	No
9.000 vs. 4.000	1.699	3.220	0.00180	0.001	No
10.000 vs. 13.000	1.695	3.211	0.00185	0.001	No
18.000 vs. 1.000	1.643	3.113	0.00251	0.001	No
4.000 vs. 5.000	1.636	3.099	0.00262	0.001	No
10.000 vs. 15.000	1.581	2.995	0.00358	0.001	No
8.000 vs. 18.000	1.573	2.980	0.00374	0.001	No
14.000 vs. 13.000	1.563	2.961	0.00395	0.001	No
16.000 vs. 5.000	1.555	2.946	0.00413	0.001	No
12.000 vs. 2.000	1.544	2.924	0.00440	0.001	No
7.000 vs. 8.000	1.867	2.888	0.00490	0.001	No
4.000 vs. 3.000	1.472	2.789	0.00649	0.001	No
1.000 vs. 6.000	1.450	2.747	0.00730	0.001	No
14.000 vs. 15.000	1.449	2.745	0.00735	0.001	No
8.000 vs. 17.000	1.419	2.688	0.00862	0.001	No
9.000 vs. 12.000	1.412	2.675	0.00893	0.001	No
16.000 vs. 3.000	1.391	2.636	0.00995	0.001	No
10.000 vs. 16.000	1.338	2.535	0.0130	0.001	No
17.000 vs. 13.000	1.336	2.531	0.0132	0.001	No

15.000 vs. 5.000	1.312	2.486	0.0148	0.001	No
11.000 vs. 18.000	1.283	2.431	0.0171	0.001	No
10.000 vs. 4.000	1.257	2.382	0.0194	0.001	No
4.000 vs. 2.000	1.256	2.379	0.0195	0.001	No
17.000 vs. 15.000	1.222	2.315	0.0230	0.001	No
14.000 vs. 16.000	1.206	2.285	0.0247	0.001	No
13.000 vs. 5.000	1.198	2.270	0.0257	0.001	No
8.000 vs. 14.000	1.192	2.258	0.0265	0.001	No
12.000 vs. 1.000	1.187	2.248	0.0271	0.001	No
18.000 vs. 13.000	1.182	2.239	0.0277	0.001	No
16.000 vs. 2.000	1.175	2.226	0.0286	0.001	No
15.000 vs. 3.000	1.148	2.176	0.0323	0.001	No
11.000 vs. 17.000	1.129	2.139	0.0353	0.001	No
14.000 vs. 4.000	1.125	2.132	0.0359	0.001	No
2.000 vs. 6.000	1.093	2.071	0.0413	0.001	No
18.000 vs. 15.000	1.068	2.023	0.0462	0.001	No
8.000 vs. 10.000	1.060	2.008	0.0478	0.001	No
13.000 vs. 3.000	1.034	1.960	0.0533	0.001	No
17.000 vs. 16.000	0.979	1.855	0.0669	0.001	No
10.000 vs. 12.000	0.970	1.837	0.0697	0.001	No
9.000 vs. 18.000	0.955	1.810	0.0737	0.001	No
15.000 vs. 2.000	0.932	1.766	0.0809	0.001	No
11.000 vs. 14.000	0.902	1.709	0.0911	0.001	No
4.000 vs. 1.000	0.899	1.703	0.0921	0.001	No
17.000 vs. 4.000	0.898	1.702	0.0924	0.001	No
3.000 vs. 6.000	0.877	1.662	0.100	0.001	No
14.000 vs. 12.000	0.837	1.587	0.116	0.001	No
18.000 vs.	0.825	1.563	0.122	0.001	No

16.000					
13.000 vs. 2.000	0.818	1.550	0.125	0.001	No
16.000 vs. 1.000	0.818	1.550	0.125	0.001	No
9.000 vs. 17.000	0.801	1.518	0.133	0.001	No
11.000 vs. 10.000	0.770	1.459	0.148	0.002	No
18.000 vs. 4.000	0.744	1.409	0.162	0.002	No
1.000 vs. 5.000	0.737	1.396	0.166	0.002	No
12.000 vs. 13.000	0.726	1.374	0.173	0.002	No
5.000 vs. 6.000	0.713	1.352	0.180	0.002	No
8.000 vs. 9.000	0.618	1.170	0.245	0.002	No
12.000 vs. 15.000	0.611	1.158	0.250	0.002	No
17.000 vs. 12.000	0.611	1.157	0.250	0.002	No
15.000 vs. 1.000	0.575	1.090	0.279	0.002	No
9.000 vs. 14.000	0.574	1.088	0.280	0.002	No
1.000 vs. 3.000	0.573	1.086	0.281	0.002	No
10.000 vs. 18.000	0.513	0.972	0.334	0.002	No
13.000 vs. 1.000	0.461	0.874	0.385	0.002	No
18.000 vs. 12.000	0.456	0.865	0.390	0.002	No
9.000 vs. 10.000	0.442	0.838	0.404	0.003	No
4.000 vs. 13.000	0.438	0.829	0.409	0.003	No
14.000 vs. 18.000	0.381	0.722	0.472	0.003	No
2.000 vs. 5.000	0.380	0.720	0.474	0.003	No
12.000 vs. 16.000	0.369	0.698	0.487	0.003	No
10.000 vs. 17.000	0.359	0.680	0.498	0.003	No
1.000 vs. 2.000	0.357	0.676	0.501	0.004	No
16.000 vs. 13.000	0.357	0.676	0.501	0.004	No
11.000 vs. 9.000	0.328	0.621	0.536	0.004	No
4.000 vs. 15.000	0.324	0.613	0.541	0.005	No

8.000 vs. 11.000	0.290	0.549	0.584	0.005	No
12.000 vs. 4.000	0.288	0.545	0.587	0.006	No
16.000 vs. 15.000	0.243	0.460	0.647	0.006	No
14.000 vs. 17.000	0.227	0.430	0.668	0.007	No
2.000 vs. 3.000	0.216	0.410	0.683	0.009	No
3.000 vs. 5.000	0.164	0.310	0.757	0.010	No
17.000 vs. 18.000	0.154	0.292	0.771	0.013	No
10.000 vs. 14.000	0.132	0.250	0.803	0.017	No
15.000 vs. 13.000	0.114	0.216	0.829	0.025	No
4.000 vs. 16.000	0.0810	0.153	0.878	0.050	No

Appendix 13: Table of the results of descriptive statistical tests on bone section KAS2 and RPT

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	144	0	-16.557	0.915	0.0763
Oxygen Value	144	0	-6.662	1.180	0.0983

Column	Range	Max	Min	Median
Carbon Value	4.292	-13.583	-17.875	-16.848
Oxygen Value	5.541	-3.045	-8.586	-6.838

Appendix 14: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section KAS2 and RPT

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	12	0	-15.772	1.322	0.382
2.000	12	0	-16.479	0.952	0.275
3.000	12	0	-17.216	0.286	0.0827
4.000	12	0	-17.205	0.488	0.141
6.000	12	0	-16.803	0.721	0.208
7.000	12	0	-16.423	0.291	0.0840
8.000	12	0	-16.567	0.686	0.198
9.000	12	0	-16.841	0.701	0.202

Source of Variation	DF	SS	MS	F	P
Between Groups	7	18.536	2.648	4.666	<0.001
Residual	88	49.939	0.567		
Total	95	68.474			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 3.000	1.444	4.694	0.00000979	0.002	Yes
1.000 vs. 4.000	1.433	4.659	0.0000112	0.002	Yes
1.000 vs. 9.000	1.069	3.476	0.000793	0.002	Yes
1.000 vs. 6.000	1.031	3.352	0.00119	0.002	Yes
1.000 vs. 8.000	0.795	2.584	0.0114	0.002	No
7.000 vs. 3.000	0.793	2.577	0.0116	0.002	No
7.000 vs. 4.000	0.782	2.542	0.0128	0.002	No
2.000 vs. 3.000	0.736	2.395	0.0188	0.002	No
2.000 vs. 4.000	0.726	2.360	0.0205	0.003	No
1.000 vs. 2.000	0.707	2.299	0.0239	0.003	No
1.000 vs. 7.000	0.651	2.116	0.0371	0.003	No
8.000 vs. 3.000	0.649	2.110	0.0377	0.003	No
8.000 vs. 4.000	0.638	2.075	0.0409	0.003	No
7.000 vs. 9.000	0.418	1.359	0.177	0.003	No
6.000 vs. 3.000	0.413	1.342	0.183	0.004	No
6.000 vs. 4.000	0.402	1.307	0.195	0.004	No
7.000 vs. 6.000	0.380	1.235	0.220	0.004	No
9.000 vs. 3.000	0.375	1.218	0.226	0.005	No
9.000 vs. 4.000	0.364	1.183	0.240	0.005	No
2.000 vs. 9.000	0.362	1.177	0.242	0.006	No
2.000 vs. 6.000	0.324	1.053	0.295	0.006	No
8.000 vs. 9.000	0.274	0.892	0.375	0.007	No
8.000 vs. 6.000	0.236	0.768	0.445	0.009	No
7.000 vs. 8.000	0.144	0.468	0.641	0.010	No
2.000 vs. 8.000	0.0877	0.285	0.776	0.013	No
7.000 vs. 2.000	0.0562	0.183	0.856	0.017	No
6.000 vs.	0.0382	0.124	0.902	0.025	No

9.000					
4.000 vs. 3.000	0.0108	0.0350	0.972	0.050	No

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	12	0	-4.680	1.110	0.321
2.000	12	0	-6.743	1.080	0.312
3.000	12	0	-6.558	0.733	0.212
4.000	12	0	-6.966	0.588	0.170
6.000	12	0	-7.318	0.583	0.168
7.000	12	0	-7.303	0.616	0.178
8.000	12	0	-6.981	0.758	0.219
9.000	12	0	-7.169	0.628	0.181

Source of Variation	DF	SS	MS	F	P
Between Groups	7	62.581	8.940	14.390	<0.001
Residual	88	54.673	0.621		
Total	95	117.254			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 6.000	2.638	8.197	1.834E-012	0.002	Yes
1.000 vs. 7.000	2.623	8.151	2.279E-012	0.002	Yes
1.000 vs. 9.000	2.489	7.733	1.618E-011	0.002	Yes
1.000 vs. 8.000	2.301	7.152	0.000000000241	0.002	Yes
1.000 vs. 4.000	2.286	7.104	0.000000000301	0.002	Yes
1.000 vs. 2.000	2.063	6.410	0.00000000701	0.002	Yes
1.000 vs. 3.000	1.878	5.836	0.0000000877	0.002	Yes
3.000 vs. 6.000	0.760	2.361	0.0204	0.002	No
3.000 vs. 7.000	0.745	2.315	0.0229	0.003	No
3.000 vs. 9.000	0.611	1.898	0.0610	0.003	No
2.000 vs. 6.000	0.575	1.787	0.0774	0.003	No
2.000 vs. 7.000	0.560	1.741	0.0852	0.003	No
2.000 vs. 9.000	0.426	1.323	0.189	0.003	No
3.000 vs. 8.000	0.424	1.317	0.191	0.003	No
3.000 vs. 4.000	0.408	1.268	0.208	0.004	No
4.000 vs.	0.352	1.093	0.277	0.004	No

6.000					
4.000 vs. 7.000	0.337	1.047	0.298	0.004	No
8.000 vs. 6.000	0.336	1.045	0.299	0.005	No
8.000 vs. 7.000	0.321	0.999	0.321	0.005	No
2.000 vs. 8.000	0.239	0.742	0.460	0.006	No
2.000 vs. 4.000	0.223	0.694	0.490	0.006	No
4.000 vs. 9.000	0.203	0.630	0.531	0.007	No
8.000 vs. 9.000	0.187	0.581	0.563	0.009	No
3.000 vs. 2.000	0.185	0.574	0.567	0.010	No
9.000 vs. 6.000	0.149	0.464	0.644	0.013	No
9.000 vs. 7.000	0.134	0.417	0.677	0.017	No
4.000 vs. 8.000	0.0156	0.0484	0.961	0.025	No
7.000 vs. 6.000	0.0148	0.0461	0.963	0.050	No

Appendix 15: Table of the results of descriptive statistical tests on bone section 792L

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	48	0	-12.917	0.505	0.0729
Oxygen Value	48	0	-2.977	0.895	0.129

Column	Range	Max	Min	Median
Carbon Value	1.741	-11.989	-13.730	-12.896
Oxygen Value	3.590	-0.725	-4.315	-3.096

Appendix 16: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section 792L

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-12.361	0.278	0.113
2.000	6	0	-12.833	0.139	0.0566
3.000	6	0	-13.398	0.197	0.0803
4.000	6	0	-13.494	0.139	0.0569
5.000	6	0	-12.465	0.482	0.197
6.000	6	0	-13.490	0.143	0.0584
7.000	6	0	-12.819	0.177	0.0721
8.000	6	0	-12.476	0.143	0.0583

Source of Variation	DF	SS	MS	F	P
Between Groups	7	9.700	1.386	24.154	<0.001
Residual	40	2.295	0.0574		
Total	47	11.994			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 4.000	1.133	8.192	0.000000000435	0.002	Yes
1.000 vs. 6.000	1.129	8.163	0.000000000476	0.002	Yes
1.000 vs. 3.000	1.037	7.498	0.00000000384	0.002	Yes
5.000 vs. 4.000	1.028	7.437	0.00000000465	0.002	Yes
5.000 vs. 6.000	1.024	7.409	0.00000000509	0.002	Yes
8.000 vs. 4.000	1.018	7.363	0.00000000589	0.002	Yes
8.000 vs. 6.000	1.014	7.334	0.00000000646	0.002	Yes
5.000 vs. 3.000	0.932	6.743	0.0000000428	0.002	Yes
8.000 vs. 3.000	0.922	6.669	0.0000000544	0.003	Yes
7.000 vs. 4.000	0.675	4.878	0.0000175	0.003	Yes
7.000 vs. 6.000	0.670	4.849	0.0000192	0.003	Yes
2.000 vs. 4.000	0.661	4.780	0.0000238	0.003	Yes
2.000 vs. 6.000	0.657	4.751	0.0000261	0.003	Yes
7.000 vs. 3.000	0.578	4.183	0.000152	0.003	Yes
2.000 vs. 3.000	0.565	4.086	0.000205	0.004	Yes
1.000 vs. 2.000	0.472	3.412	0.00149	0.004	Yes
1.000 vs. 7.000	0.458	3.314	0.00196	0.004	Yes
5.000 vs. 2.000	0.367	2.658	0.0113	0.005	No
8.000 vs. 2.000	0.357	2.583	0.0136	0.005	No
5.000 vs. 7.000	0.354	2.560	0.0144	0.006	No
8.000 vs. 7.000	0.344	2.485	0.0172	0.006	No
1.000 vs. 8.000	0.115	0.829	0.412	0.007	No
1.000 vs. 5.000	0.104	0.754	0.455	0.009	No
3.000 vs. 4.000	0.0960	0.694	0.492	0.010	No
3.000 vs. 6.000	0.0920	0.665	0.510	0.013	No
7.000 vs. 2.000	0.0135	0.0976	0.923	0.017	No

5.000 vs. 8.000	0.0103	0.0747	0.941	0.025	No
6.000 vs. 4.000	0.00400	0.0289	0.977	0.050	No

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-2.620	1.282	0.523
2.000	6	0	-2.599	0.320	0.130
3.000	6	0	-3.246	0.289	0.118
4.000	6	0	-2.371	0.587	0.240
5.000	6	0	-3.243	1.181	0.482
6.000	6	0	-3.235	0.604	0.247
7.000	6	0	-2.688	1.261	0.515
8.000	6	0	-3.812	0.247	0.101

Source of Variation	DF	SS	MS	F	P
Between Groups	7	9.771	1.396	2.000	0.079
Residual	40	27.916	0.698		
Total	47	37.686			

Appendix 17: Table of the results of descriptive statistical tests on bone section 792R

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	48	0	-12.864	0.376	0.0542
Oxygen Value	48	0	-2.825	0.911	0.131

Column	Range	Max	Min	Median
Carbon Value	1.854	-11.773	-13.627	-12.834
Oxygen Value	4.026	-0.493	-4.519	-2.849

Appendix 18: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section 792R

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-12.793	0.245	0.100
2.000	6	0	-12.810	0.736	0.301
3.000	6	0	-12.790	0.177	0.0724
4.000	6	0	-13.022	0.305	0.124
5.000	6	0	-12.894	0.617	0.252
6.000	6	0	-12.810	0.206	0.0841
7.000	6	0	-12.695	0.159	0.0651
8.000	6	0	-13.096	0.0419	0.0171

Source of Variation	DF	SS	MS	F	P
Between Groups	7	0.747	0.107	0.725	0.652
Residual	40	5.883	0.147		
Total	47	6.630			

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-1.638	1.050	0.429
2.000	6	0	-2.816	0.662	0.270
3.000	6	0	-2.463	0.384	0.157
4.000	6	0	-2.638	0.791	0.323
5.000	6	0	-2.668	0.591	0.241
6.000	6	0	-2.652	0.313	0.128
7.000	6	0	-3.536	0.446	0.182
8.000	6	0	-4.188	0.225	0.0917

Source of Variation	DF	SS	MS	F	P
Between Groups	7	23.952	3.422	9.093	<0.001
Residual	40	15.052	0.376		
Total	47	39.004			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 8.000	2.550	7.199	0.00000000992	0.002	Yes
1.000 vs. 7.000	1.898	5.359	0.00000375	0.002	Yes
3.000 vs. 8.000	1.725	4.870	0.0000179	0.002	Yes
4.000 vs. 8.000	1.550	4.376	0.0000844	0.002	Yes
6.000 vs. 8.000	1.536	4.336	0.0000954	0.002	Yes
5.000 vs. 8.000	1.520	4.292	0.000109	0.002	Yes
2.000 vs. 8.000	1.371	3.872	0.000390	0.002	Yes
1.000 vs. 2.000	1.178	3.327	0.00189	0.002	Yes
3.000 vs. 7.000	1.073	3.030	0.00428	0.003	No
1.000 vs. 5.000	1.030	2.907	0.00592	0.003	No
1.000 vs. 6.000	1.014	2.863	0.00665	0.003	No
1.000 vs. 4.000	1.000	2.824	0.00737	0.003	No
4.000 vs. 7.000	0.898	2.535	0.0153	0.003	No
6.000 vs. 7.000	0.884	2.496	0.0168	0.003	No
5.000 vs. 7.000	0.868	2.451	0.0187	0.004	No
1.000 vs. 3.000	0.825	2.329	0.0250	0.004	No
2.000 vs. 7.000	0.720	2.032	0.0489	0.004	No
7.000 vs.	0.652	1.840	0.0731	0.005	No

8.000					
3.000 vs. 2.000	0.353	0.998	0.324	0.005	No
3.000 vs. 5.000	0.205	0.578	0.566	0.006	No
3.000 vs. 6.000	0.189	0.534	0.596	0.006	No
4.000 vs. 2.000	0.178	0.504	0.617	0.007	No
3.000 vs. 4.000	0.175	0.495	0.624	0.009	No
6.000 vs. 2.000	0.164	0.464	0.645	0.010	No
5.000 vs. 2.000	0.149	0.420	0.677	0.013	No
4.000 vs. 5.000	0.0297	0.0838	0.934	0.017	No
6.000 vs. 5.000	0.0157	0.0442	0.965	0.025	No
4.000 vs. 6.000	0.0140	0.0395	0.969	0.050	No

Appendix 19: Table of the results of descriptive statistical tests on bone section UoD

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	48	0	-14.822	4.503	0.650
Oxygen Value	48	0	-2.922	1.488	0.215

Column	Range	Max	Min	Median
Carbon Value	31.851	15.654	-16.197	-15.461
Oxygen Value	8.270	3.000	-5.270	-2.767

Appendix 20: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section UoD

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-15.583	0.437	0.179
2.000	3	0	-15.512	0.0913	0.0527
3.000	3	0	-15.873	0.105	0.0605
4.000	3	0	-15.638	0.176	0.102
5.000	3	0	-15.494	0.180	0.104
6.000	3	0	-15.680	0.0478	0.0276
7.000	3	0	-15.570	0.0147	0.00850
8.000	3	0	-15.708	0.149	0.0862
9.000	3	0	-15.765	0.117	0.0674
10.000	3	0	-15.530	0.112	0.0646
11.000	3	0	-15.589	0.181	0.104
12.000	3	0	-15.776	0.0885	0.0511
13.000	3	0	-15.640	0.0238	0.0137
14.000	3	0	-15.766	0.348	0.201
15.000	3	0	-16.061	0.0816	0.0471

Source of Variation	DF	SS	MS	F	P

Between Groups	14	1.004	0.0717	1.516	0.160
Residual	33	1.562	0.0473		
Total	47	2.566			

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-1.919	0.300	0.122
2.000	3	0	-3.508	0.278	0.161
3.000	3	0	-3.659	0.570	0.329
4.000	3	0	-4.089	0.540	0.312
5.000	3	0	-4.546	0.140	0.0806
6.000	3	0	-5.087	0.174	0.100
7.000	3	0	-4.684	0.0770	0.0445
8.000	3	0	-4.525	0.262	0.151
9.000	3	0	-5.300	0.284	0.164
10.000	3	0	-5.265	0.482	0.278
11.000	3	0	-5.353	0.363	0.210
12.000	3	0	-5.126	0.473	0.273
13.000	3	0	-4.554	0.696	0.402
14.000	3	0	-5.548	0.565	0.326
15.000	3	0	-3.216	0.156	0.0899

Source of Variation	DF	SS	MS	F	P
Between Groups	14	59.405	4.243	27.575	<0.001
Residual	33	5.078	0.154		
Total	47	64.483			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 14.000	3.629	13.082	1.308E-014	0.000	Yes
1.000 vs. 11.000	3.434	12.381	5.981E-014	0.000	Yes
1.000 vs. 9.000	3.381	12.190	9.138E-014	0.000	Yes
1.000 vs. 10.000	3.346	12.062	1.215E-013	0.001	Yes
1.000 vs. 12.000	3.208	11.564	3.777E-013	0.001	Yes
1.000 vs. 6.000	3.168	11.421	5.259E-013	0.001	Yes
1.000 vs. 7.000	2.765	9.969	1.750E-011	0.001	Yes
1.000 vs. 13.000	2.635	9.499	5.771E-011	0.001	Yes
1.000 vs. 5.000	2.627	9.470	6.216E-011	0.001	Yes
1.000 vs. 8.000	2.606	9.396	7.532E-011	0.001	Yes
1.000 vs. 4.000	2.170	7.823	0.00000000514	0.001	Yes
15.000 vs. 14.000	2.331	7.279	0.0000000237	0.001	Yes

15.000 vs. 11.000	2.137	6.671	0.000000136	0.001	Yes
15.000 vs. 9.000	2.084	6.506	0.000000220	0.001	Yes
15.000 vs. 10.000	2.048	6.395	0.000000303	0.001	Yes
2.000 vs. 14.000	2.040	6.368	0.000000328	0.001	Yes
1.000 vs. 3.000	1.740	6.274	0.000000432	0.001	Yes
15.000 vs. 12.000	1.910	5.963	0.00000107	0.001	Yes
3.000 vs. 14.000	1.889	5.897	0.00000131	0.001	Yes
15.000 vs. 6.000	1.870	5.839	0.00000155	0.001	Yes
2.000 vs. 11.000	1.845	5.760	0.00000195	0.001	Yes
1.000 vs. 2.000	1.589	5.729	0.00000214	0.001	Yes
2.000 vs. 9.000	1.792	5.595	0.00000319	0.001	Yes
2.000 vs. 10.000	1.757	5.485	0.00000441	0.001	Yes
3.000 vs. 11.000	1.694	5.289	0.00000787	0.001	Yes
3.000 vs. 9.000	1.641	5.123	0.0000128	0.001	Yes
2.000 vs. 12.000	1.618	5.053	0.0000158	0.001	Yes
3.000 vs. 10.000	1.606	5.013	0.0000177	0.001	Yes
2.000 vs. 6.000	1.579	4.929	0.0000227	0.001	Yes
1.000 vs. 15.000	1.298	4.678	0.0000475	0.001	Yes
15.000 vs. 7.000	1.468	4.582	0.0000628	0.001	Yes
3.000 vs. 12.000	1.467	4.581	0.0000630	0.001	Yes
4.000 vs. 14.000	1.459	4.555	0.0000680	0.001	Yes
3.000 vs. 6.000	1.428	4.457	0.0000904	0.001	Yes
15.000 vs. 13.000	1.337	4.175	0.000204	0.001	Yes
15.000 vs. 5.000	1.329	4.150	0.000219	0.001	Yes
15.000 vs. 8.000	1.309	4.086	0.000264	0.001	Yes
4.000 vs. 11.000	1.264	3.947	0.000391	0.001	Yes
4.000 vs. 9.000	1.211	3.782	0.000622	0.001	Yes
4.000 vs.	1.176	3.672	0.000845	0.001	No

10.000					
2.000 vs. 7.000	1.176	3.672	0.000845	0.001	No
2.000 vs. 13.000	1.046	3.265	0.00256	0.001	No
4.000 vs. 12.000	1.038	3.240	0.00273	0.001	No
2.000 vs. 5.000	1.038	3.240	0.00273	0.001	No
3.000 vs. 7.000	1.025	3.200	0.00303	0.001	No
8.000 vs. 14.000	1.023	3.193	0.00309	0.001	No
2.000 vs. 8.000	1.017	3.175	0.00324	0.001	No
5.000 vs. 14.000	1.002	3.128	0.00366	0.001	No
4.000 vs. 6.000	0.998	3.116	0.00378	0.001	No
13.000 vs. 14.000	0.994	3.103	0.00391	0.001	No
3.000 vs. 13.000	0.895	2.793	0.00862	0.001	No
3.000 vs. 5.000	0.887	2.768	0.00917	0.001	No
15.000 vs. 4.000	0.872	2.724	0.0102	0.001	No
3.000 vs. 8.000	0.866	2.704	0.0108	0.001	No
7.000 vs. 14.000	0.864	2.696	0.0109	0.001	No
8.000 vs. 11.000	0.828	2.585	0.0143	0.001	No
5.000 vs. 11.000	0.807	2.521	0.0167	0.001	No
13.000 vs. 11.000	0.799	2.496	0.0177	0.001	No
8.000 vs. 9.000	0.775	2.420	0.0212	0.001	No
5.000 vs. 9.000	0.754	2.355	0.0246	0.001	No
13.000 vs. 9.000	0.746	2.330	0.0261	0.001	No
8.000 vs. 10.000	0.740	2.309	0.0273	0.001	No
5.000 vs. 10.000	0.719	2.245	0.0316	0.001	No
13.000 vs. 10.000	0.711	2.220	0.0334	0.001	No
7.000 vs. 11.000	0.669	2.089	0.0445	0.001	No
7.000 vs. 9.000	0.616	1.923	0.0631	0.001	No
8.000 vs. 12.000	0.601	1.877	0.0693	0.001	No

4.000 vs. 7.000	0.595	1.859	0.0720	0.001	No
5.000 vs. 12.000	0.581	1.813	0.0789	0.001	No
7.000 vs. 10.000	0.581	1.813	0.0789	0.001	No
2.000 vs. 4.000	0.581	1.813	0.0789	0.001	No
13.000 vs. 12.000	0.573	1.788	0.0830	0.002	No
8.000 vs. 6.000	0.562	1.754	0.0888	0.002	No
5.000 vs. 6.000	0.541	1.689	0.101	0.002	No
13.000 vs. 6.000	0.533	1.664	0.106	0.002	No
4.000 vs. 13.000	0.465	1.452	0.156	0.002	No
6.000 vs. 14.000	0.461	1.439	0.159	0.002	No
4.000 vs. 5.000	0.457	1.427	0.163	0.002	No
15.000 vs. 3.000	0.443	1.382	0.176	0.002	No
7.000 vs. 12.000	0.442	1.381	0.177	0.002	No
4.000 vs. 8.000	0.436	1.362	0.182	0.002	No
3.000 vs. 4.000	0.430	1.341	0.189	0.002	No
12.000 vs. 14.000	0.421	1.315	0.197	0.002	No
7.000 vs. 6.000	0.403	1.257	0.218	0.002	No
15.000 vs. 2.000	0.292	0.911	0.369	0.002	No
10.000 vs. 14.000	0.283	0.884	0.383	0.003	No
6.000 vs. 11.000	0.266	0.832	0.412	0.003	No
9.000 vs. 14.000	0.248	0.773	0.445	0.003	No
12.000 vs. 11.000	0.227	0.708	0.484	0.003	No
6.000 vs. 9.000	0.213	0.666	0.510	0.003	No
11.000 vs. 14.000	0.195	0.608	0.547	0.003	No
6.000 vs. 10.000	0.178	0.556	0.582	0.004	No
12.000 vs. 9.000	0.174	0.542	0.591	0.004	No
8.000 vs. 7.000	0.159	0.496	0.623	0.004	No
2.000 vs.	0.151	0.471	0.640	0.005	No

3.000					
5.000 vs. 7.000	0.138	0.432	0.669	0.005	No
12.000 vs. 10.000	0.138	0.432	0.669	0.006	No
13.000 vs. 7.000	0.130	0.407	0.687	0.006	No
10.000 vs. 11.000	0.0883	0.276	0.784	0.007	No
9.000 vs. 11.000	0.0530	0.165	0.870	0.009	No
6.000 vs. 12.000	0.0397	0.124	0.902	0.010	No
10.000 vs. 9.000	0.0353	0.110	0.913	0.013	No
8.000 vs. 13.000	0.0287	0.0895	0.929	0.017	No
8.000 vs. 5.000	0.0207	0.0645	0.949	0.025	No
5.000 vs. 13.000	0.00800	0.0250	0.980	0.050	No

Appendix 21: Table of the results of descriptive statistical tests on bone section 820R

Column	Size	Missing	Mean	Std Dev	Std. Error
Carbon Value	48	0	-14.822	4.503	0.650
Oxygen Value	48	0	-2.922	1.488	0.215

Column	Range	Max	Min	Median
Carbon Value	31.851	15.654	-16.197	-15.461
Oxygen Value	8.270	3.000	-5.270	-2.767

Appendix 22: Results of a one-way ANOVA performed on the $\delta^{13}C$ and $\delta^{18}O$ values from all holes in bone section 820R

Dependent Variable: Carbon Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-15.405	0.286	0.117
2.000	6	0	-15.401	0.156	0.0637
3.000	6	0	-15.130	0.118	0.0484
4.000	6	0	-15.218	0.233	0.0950
5.000	6	0	-15.567	0.137	0.0561
6.000	6	0	-10.600	12.864	5.252
7.000	6	0	-15.826	0.245	0.1000
8.000	6	0	-15.427	0.173	0.0707

Source of Variation	DF	SS	MS	F	P
Between Groups	7	124.080	17.726	0.855	0.549
Residual	40	828.869	20.722		
Total	47	952.949			

Dependent Variable: Oxygen Value

Group Name	N	Missing	Mean	Std Dev	SEM
1.000	6	0	-1.114	0.992	0.405
2.000	6	0	-1.746	2.367	0.966
3.000	6	0	-2.468	0.215	0.0878
4.000	6	0	-2.734	0.515	0.210
5.000	6	0	-4.943	0.335	0.137
6.000	6	0	-2.767	0.316	0.129
7.000	6	0	-4.250	0.275	0.112
8.000	6	0	-3.357	0.698	0.285

Source of Variation	DF	SS	MS	F	P
Between Groups	7	65.712	9.387	9.786	<0.001
Residual	40	38.371	0.959		
Total	47	104.082			

Comparison	Diff of Means	t	Unadjusted P	Critical Level	Significant?
1.000 vs. 5.000	3.829	6.771	0.0000000391	0.002	Yes
2.000 vs. 5.000	3.197	5.653	0.00000145	0.002	Yes
1.000 vs. 7.000	3.136	5.545	0.00000206	0.002	Yes
2.000 vs. 7.000	2.503	4.427	0.0000720	0.002	Yes
3.000 vs. 5.000	2.475	4.376	0.0000842	0.002	Yes
1.000 vs. 8.000	2.243	3.966	0.000294	0.002	Yes
4.000 vs. 5.000	2.209	3.906	0.000352	0.002	Yes
6.000 vs. 5.000	2.176	3.848	0.000419	0.002	Yes
3.000 vs. 7.000	1.781	3.150	0.00308	0.003	No
1.000 vs. 6.000	1.653	2.923	0.00568	0.003	No
1.000 vs. 4.000	1.620	2.865	0.00662	0.003	No
2.000 vs. 8.000	1.611	2.848	0.00691	0.003	No
8.000 vs. 5.000	1.586	2.805	0.00774	0.003	No
4.000 vs. 7.000	1.516	2.680	0.0106	0.003	No
6.000 vs. 7.000	1.483	2.622	0.0123	0.004	No
1.000 vs. 3.000	1.354	2.395	0.0214	0.004	No
2.000 vs. 6.000	1.021	1.805	0.0786	0.004	No
2.000 vs. 4.000	0.988	1.747	0.0884	0.005	No

8.000 vs. 7.000	0.893	1.579	0.122	0.005	No
3.000 vs. 8.000	0.889	1.572	0.124	0.006	No
2.000 vs. 3.000	0.722	1.277	0.209	0.006	No
7.000 vs. 5.000	0.693	1.226	0.227	0.007	No
1.000 vs. 2.000	0.632	1.118	0.270	0.009	No
4.000 vs. 8.000	0.623	1.102	0.277	0.010	No
6.000 vs. 8.000	0.590	1.043	0.303	0.013	No
3.000 vs. 6.000	0.299	0.528	0.600	0.017	No
3.000 vs. 4.000	0.266	0.470	0.641	0.025	No
4.000 vs. 6.000	0.0332	0.0587	0.954	0.050	No

Appendix 23: Table demonstrating the results of ^{18}O stable isotope analysis of tap water samples collected from Dundee

Group Name	Size	Mean	Std Dev
$\delta^{18}O$	62	-7.631	1.14